People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

Echahid Hamma Lakhdar University of El-Oued

Faculty of Natural and Life Sciences

Department of Cellular and Molecular Biology

Master's Thesis

In order obtain a diploma of an Academic Master

In biological sciences

Specialty: Applied Biochemistry

Theme

Study of bioresponsive copper oxide nanoparticles as a vaccine delivery material against scorpion envenoming

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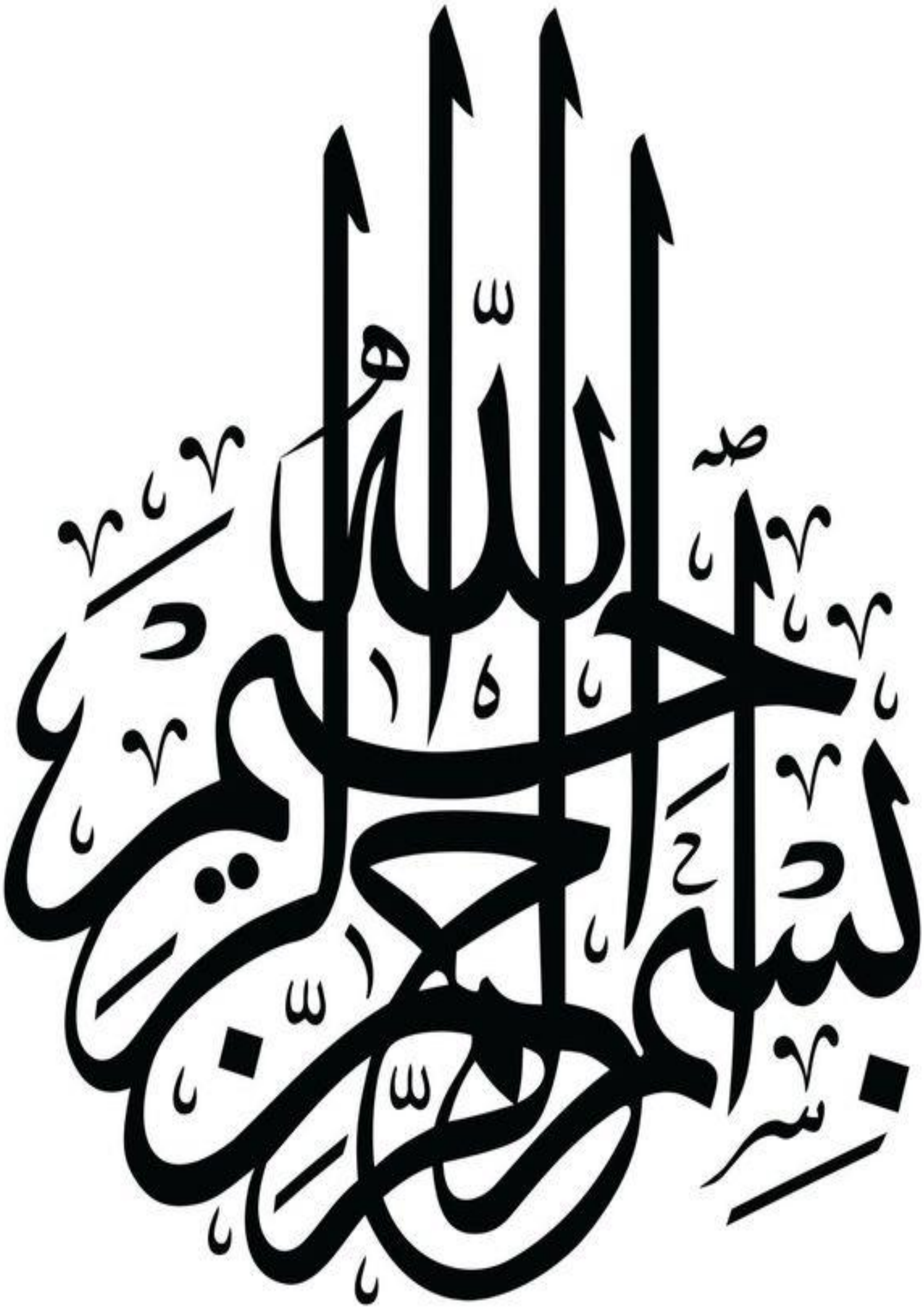
University year: 2024/2025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إهـــــــــداء

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قال تعالى:

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ)

أهدي ثمرة جهدي إلى التي حمتني ومنحتني الحياة وأحاطتني بحنانها وحرصت على تعليمي بصبرها وتضحيتها إلى من كان دعاؤها سر نجاحي

أمي الغالية حفظها الله

إلى الذي دعمني في مشواري الدراسي وكان وراء كل خطوة خطوتها في طريق العلم والمعرفة

أبي الغالي رعاه الله

. أتمنى أن يكون هذا العمل المتواضع ثمرة جهودكم التي لا تعد ولا تحصى.

إلى إخوتي

الذين لم يتركوني أبدًا، أنتم مميزون جدًا بالنسبة لي. لن أتمكن أبدًا من التغلب على الصعوبات والتركيز على دراستي بدون جهودكم المخلصة ودعمكم الثابت الذي ألهمني للتفوق

شكرا جزيلا مع الحب العميق...

الى **أستاذي ومؤطري البروفيسور درويش سمير** فله جميل الشكر على ما قدم لنا من تشجيع ودعم ونصائح طوال هذا العمل لقد كانَ المعلم والموجّه والداعم الحقيقي بارك الله لك في أعمالك واطال في عمرك وزادك من علمه الواسع.

أتوجه بالشكر والامتنان **لرفيقة الدرب اية** التي كانت نعم الاخت والسند منذ البداية

واشكر **عمي الغالي واخوالي الغاليين** على قلبي على دعمهم المستمر لي طوال حياتي الدراسية

. وأتوجه بالشكر أيضًا إلى كل أولئك الذين قدموا لي يومًا أو آخر صداقتهم ولحظاتهم التي لا تُنسى طوال دراستي الجامعية

اهديكم بحث تخرجي هذا متمنيه من الله عز وجل القبول والرضا وداعيتا إياه ان يطيل في اعماركم ويرزقكم من خيراته الرزق الوفير في الدنيا والفردوس الأعلى في الاخرة

إيـــــــمان

إهـــــــــــداء

أحمد الله الذي أكرمني بهذه اللحظة التي حلمت بها طويلًا، وجعلها مكلّلة بالفخر والامتنان.

إلى أمي الحبيبة،

مصدر الحنان، ودعاؤها كان نوري في طريق التعب.

إلى أبي العزيز،

سندي الأول، وكلماته كانت دافعًا، ودعاؤه زادي في كل خطوة.

إلى إخوتي وأخواتي،

أنتم حصني ودفئي، ووجودكم كان عزائي في كل الأوقات.

إلى أستاذي الفاضل البروفيسور درويش سمير،

من حمل شعلة العلم وأنار بها دربي، شكرًا لصبرك، دعمك، وتوجيهك الذي رافقني طيلة مشواري الجامعي. لقد كنتَ المعلم والموجّه والداعم الحقيقي،

فلك مني أسمى عبارات الامتنان والتقدير.

وإلى صديقتي الغالية إيمان،

رفيقة الدرب وشريكة الحلم، وجودك أنار طريقي، وجعل كل لحظة أجمل. هذا النجاح لك كما هو لي.

هذا التخرج ليس مجرد شهادة، بل خلاصة تعب وامتنان لكل من دعمني.

وأختم قائلة: سعيتُ فبلغت، وبفضلكم تحقق الحلم، فشكرًا لكم من القلب

آيـــــة الرحمان

Acknowledgement

First and foremost, we would like to express our deepest gratitude to Allah, the most gracious and the most merciful for granting us the strength, patience, and wisdom to complete this thesis. Without His guidance and blessings, this work would not have been possible

We express our deep gratitude to **Pr. Derouiche Samir** for supervising this work and providing valuable advice throughout

We also wish to thank the jury members, for their constructive suggestions and feedback. Their expertise has contributed to the refinement of this thesis

Special thanks to May 8 th Emergency LAB pening their doors and helping us to make this research possible. We also extend thanks to our faculty Lab; Faculty of Natural and Life Sciences- Echahid Hamma Lakhdar El-Oued University, under the supervision of Ms. GOBI Sana and the help of the Laboratory journey: REZZAG MOHCEN bouchra. We also thank everyone who contributed to the completion of this work.

To all those who have contributed to this work in any way, we extend our sincerest thanks to you.

Abstract

This study aims to biosynthesize a bio-vaccine based on copper oxide nanoparticles **(CuONPs)** using scorpion venom and investigate its protective effect and efficacy against scorpion envenoming. The biosynthesis of CuONPs was confirmed using UV-Vis spectroscopy, IR spectroscopy, scanning electron microscopy (SEM), and EDX analysis, Transmission electron microscopy (TEM), XRD, and XPS analysis. An antibacterial activity was tested. In an *in-vivo* study, 15 male Wistar albino rats were randomly divided into three groups (n = 5): control group, scorpion venom-treated group, and SV-CuONPs-vaccinated group. The vaccination was done via intraperitoneal injection at a dose of 5 mg/kg. After two weeks, the groups were challenged with a toxic dose of venom, except the control group. Some hematological, biochemical parameters were evaluated, in addition to microscopic observation of liver, spleen, and kidney tissues. Results of the *in-vitro* study showed that SV-CuONPs were spherical or sub spherical, and others were aggregated into larger clusters and size ranging from 3-10 nm. The in-vivo study results indicated significant changes caused by the venom, including physiological changes and disturbances in Hematological parameters WBC decrease ($p<0.05$), biochemical parameters Blood sugar a decrease ($p<0.05$) and antioxidant defense systems MDA a decrease ($p<0.05$) in studied organs. However, the results of rats immunized with SV-CuONPs showed a significant improvement in biological parameters and histological analysis liver, spleen, kindey. Indicating the efficacy of the nanoparticles. indicating the efficacy of the nanoparticles. These findings suggest that the SV-CuONPs bio-vaccine is effective in providing protection against scorpion envenoming with minimal side effects.

Keywords: bio-vaccine, CuONPs, scorpion venom, biosynthesis, immunitary system, Wistar rats.

الملخص

تهدف هذه الدراسة إلى تخليق لقاح حيوي قائم على جسيمات أكسيد النحاس النانوية (CuONPs) باستخدام سم العقرب، ودراسة تأثيره الوقائي وفعاليته ضد تسمم العقارب. تم تأكيد التخليق الحيوي لجسيمات أكسيد النحاس النانوية باستخدام مطيافية الأشعة فوق البنفسجية والمرئية، ومطيافية الأشعة تحت الحمراء، والمجهر الإلكتروني الماسح(SEM) ، وتحليلEDX ، والمجهر الإلكتروني النافذ(TEM) ، وتقنيةXRD ، وتقنية XPS والنشاط البكتيري في دراسة حيوية، قُسّم 15 جرذًا ذكرًا من فئران ويستار البيضاء عشوائيًا إلى ثلاث مجموعات (عددها 5): المجموعة الضابطة، والمجموعة المعالجة بسم العقرب، والمجموعة الملقحة بجسيمات أكسيد النحاس النانوية .(SV– CuONPs) أُجري التطعيم عن طريق الحقن داخل الصفاق بجرعة 5 ملغم/كغم. بعد أسبوعين، حُقنت المجموعات بجرعة سامة من السم، باستثناء المجموعة الضابطة. تم تقييم بعض المعايير الدموية والكيميائية الحيوية، بالإضافة إلى المراقبة المجهرية لأنسجة الكبد والطحال والكلى. أظهرت الدراسة المختبرية أن جزيئات SV–CuONPs كانت كروية أو شبه كروية، بينما تجمعت جزيئات أخرى في مجموعات أكبر وأحجام تتراوح بين 3 و10 نانومتر. أشارت نتائج الدراسة داخل الجسم الحي إلى تغيرات ملحوظة ناجمة عن السم، بما في ذلك تغيرات فسيولوجية واضطرابات في المعايير الدموية (انخفاض في كريات الدم البيضاء (WBCa)) (قيمة الاحتمالية <0.05)، والمعايير الكيميائية الحيوية (انخفاض في سكر الدم ($p<0.05$))، وأجهزة الدفاع المضادة للأكسدة (MDA)) (قيمة الاحتمالية <0.05)) في الأعضاء المدروسة. ومع ذلك، أظهرت نتائج الفئران المحصنة بـ SV–CuONPs تحسنًا ملحوظًا في المعايير البيولوجية والتحليل النسيجي للكبد والطحال والكلى، مما يدل على فعالية هذه الجسيمات النانوية. تشير هذه النتائج إلى أن اللقاح الحيوي SV–CuONPs فعال في توفير الحماية من تسمم العقارب مع آثار جانبية ضئيلة .

الكلمات المفتاحية: لقاح الحيوي، جزيئات النحاس النانويةCuONs ، سم العقرب، التخليق الحيوي، النظام المناعي، فئران ويستر

ABBREVIATIONS LIST

(Hb / HGB): Hemoglobin

$1O_2$: Singlet oxygen

AIDS: Acquired Immune deficiency syndrome

ALAT: Alanine Aminotransferase.

APCs: Antigen-presenting cells

APCs: Antigen-Presenting Cells

ASAT: Aspartate aminotransferase

BCG: Bacille Calmette-Guérin

CD4: Cluster of Differentiation 4

CD8: Cluster of Differentiation 8

COVID-19: Coronavirus disease 2019

CTL: Cytotoxic T Lymphocyte

CuONPs: Copper Oxide Nanoparticles

DCs: Dendritic Cells

DCs: Dendritic Cells

DIC: Disseminated Intravascular Coagulation

DNA: Deoxyribonucleic acid

DTNB: Dithio-Bis-2-Nitrobenzoic Acid

EDTA: Ethylenediaminetetraacetic acid

EDX: Energy dispersive X-ray

EM: Electromagnetic

FNS: Blood formula number

FTIR: Fourier Transform Infrared spectroscopy

GALT: Gut-Associated Lymphoid Tissue

GALT: Sociated Lymphoid Tissue

Gly: Glycemie

GSH: Reduced Glutathione

GSSG: Glutathione Disulfide

H2O2: Hydrogen peroxide

H3PO4: Orthophosphoric Acid

HCT: Hematocrit

HOCL: Hypochlorous Acid

HPA: Hypothalamic–Pituitary–Adrenal Axis

HPV: Human Papillomavirus

I.M: Intramuscular

ID: Intradermal

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IL10: Interleukin 10

IL6: Interleukin 6

IM: Intramuscular

IN: Intranasal

IP : Inhibition percentage

IPV: Inactivated poliomyelitis vaccine

IY : Immobilization yield

K2HPO4: Dipotassium phosphate

LNs: Lymph nodes

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

MCV: Mean corpuscular volume

MDA: Malondialdehyde

MHCII: Major histocompatibility complex class II

MØ: Macrophage

NACL: Sodium Chloride

NADPH2 : Reduced Nicotinamide Adenine Dinucleotide Phosphate

NALT: Nasal-Associated Lymphoid Tissue

NBT: Nitroblue Tetrazolium

NGF: Nerve Growth Factor

NH_2: Amine Group

NO: Nitric Oxide

NOX: NADPH Oxidase

NPs: Nanoparticles

O_2-: Superoxide Anion

OH·: hydroxyl radicals

PLT: Platelets

PRRs: pattern recognition receptors

RBCs: Red Blood Cells

RED: RED: Relative Energy Deficiency

RNAs: Ribonucleic Acids

RP: Red Pulp.

S.C: Subcutaneous

SE: Scropien Envenomation

SEM: Scanning Electron Microscope

SOD: Superoxide Dismutase

SSA: Specific Surface Area

SV-CuNPs: Copper Oxide Nanoparticles Synthesised By Scropien Venom

TCA: Trichloroacetic Acid

TEM: Transmission Electron Microscopy

TG: Triglyceride

TLR2: Toll-Like Receptors 2

TLR4: Toll-Like Receptors 4

TLRs: Toll-Like Receptors

TNF-α: Tumor Necrosis Factor Alpha

UV-VIS: Ultraviolet-Visible Spectroscopy

VLPs: Virus-Like Particles

WBCs: White Blood Cells

WP: White Pulp

XPS: X-ray Photoelectron Spectroscopy

XRD: X-Ray Diffraction

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Summary

Introduction

According to global public health data, about one million scorpion envenoming are recorded annually worldwide, resulting in approximately 3000 deaths (Ward et *al*., 2018). The pathology of scorpion stings ranges from mild local inflammatory reactions (Cupo, 2015) to moderate and severe envenoming causing heart failure, pulmonary edema, and pancreatitis, which may provoke lethal systemic responses.

Despite the mortality associated with their stings, scorpions have been the target of much research interest due to their observed medicinal benefits and a wide range of pharmaceutical activities. Both crude SV and its derived proteins and peptides were used in applications ranging from cosmeceuticals and diagnostics to treatment of various ailments of the cardiovascular system, convulsions, cancer (Ahmadi et *al*., 2020; Srairi-Abid et *al*. ,2019). Because of the lethality of scorpion toxins, the challenges associated with collecting the toxins, and the small amount of venom obtained from scorpions, marketing SV products for large-scale applications has been limited. Nevertheless, several toxin-based drugs have been approved and marketed over the last decade (Bordon et *al*., 2020)

Nanotechnology is the world's fastest growing technology, also known as the twenty-first-century industrial revolution. Various research, development, and manufacturing processes have been employed worldwide to create better and safer nanomaterials for a variety of uses. The phrases nanotechnology, nanoscience, and nanomaterials have gained popularity not just in scientific study, but also in everyday life (Khan et *al*., 2025). Over the last decade, nanotechnology has garnered a lot of attention from academics and practitioners, and it has played an important part in the growth of this industry. The chemical functionalization of textiles using nanotechnology has ushered in a new era of sophisticated materials with enhanced qualities and uses (Hossain et *al*., 2025). With the growing need for ecologically friendly and sustainable synthesis methods, biosynthesis (green synthesis) has gained prominence in the production of copper oxide nanoparticles. Using plants and microorganisms to generate copper oxide nanoparticles has benefits in availability, simplicity, cost-effectiveness, and environmental compatibility (Subbaiya et *al*., 2017; Akintelu & Folorunso, 2019; Subbaiya & Selvam, 2014).

our objective in this work:

In the first part of this study, the study provided an understanding of the importance of vaccines and new techniques for their development.

In the second part, it was based on the laboratory evaluation of the biosynthesis of CuONP using natural potato starch used as a carrier scorpion venom and was used as a biological vaccination against scorpion stings.

First part

Bibliographic Synthesis

Chapter I

Vaccination

I.1. Definition

Vaccination seeks to stimulate a protective immune response to a specific infectious pathogen by generating antibodies and activating certain cellular components (Canouï & Launay, 2019). This method is used in healthy people to impart protection against illness (Seneff et *al*., 2022). Vaccinations can be used to prevent disease (prophylactic vaccinations) or treat it (therapeutic vaccines) (Delany et *al*., 2014), making them one of the most effective methods for infectious disease prevention (Rambe et *al*., 2015).

I.2. Vaccine types

There are several sorts of vaccinations. Each kind teaches your immune system to combat certain pathogens and prevent dangerous infections cause (Dai et *al*., 2019). the disparities between vaccination kinds are also essential, although less well known.

Many healthcare practitioners understand this. Different Vaccines targeting the same disease may rely on Very distinct concepts as shown in Figure 1 (Paterson et *al*., 2016).

I.2.1. Live attenuated vaccines

Attenuation involves transferring a pathogen through a number of hosts, decreasing its pathogenicity. The initial creators of these approaches were unaware that the attenuation was caused by changes in virulence genes. Mutations can now be supplied randomly or targeted (Fay & Langlois, 2018; Si et al., 2022). Vaccines are great for educating the immune system to fight certain infections because They are closest to natural infections (Minor, 2015). The attenuated vaccination is temperature-sensitive to match the preferred environment of the natural pathogen.

Attenuated vaccinations provide advantages such as safety and a strong immune response from B and T cells (Giesker & Hensel, 2014).

I.2.2. Inactivated vaccines

To create vaccines, entire pathogens are inactivated using heat, radiation, or chemicals like formalin or formaldehyde. Inactivation kills the pathogens. capacity to multiply and produce the illness while maintaining Its immunogenicity enables the immune system to still recognize the intended pathogen. Inactivation methods were initially employed to make vaccinations for diseases like typhoid fever, Plague and cholera (Delany et *al*., 2014). Current examples of inactivated vaccinations include the previously mentioned IPV. Whole-cell pertussis, rabies, and hepatitis A vaccinations (Delany et *al*., 2014; Cunningham et *al*., 2016). Whole-cell pertussis vaccines are generated. Locally in several nations, using various ways; Thus, they are diverse and might evoke different Immune reaction (Edwards & Decker, 2013)

I.2.3. Subunit vaccines

use parts of the pathogen as antigens instead of the entire pathogen. These pieces may be proteins, polysaccharides, Virus components that can generate virus-like particles (VLPs). Subunit vaccinations usually induce fewer adverse reactions.

responses than live or inactivated whole-organism vaccinations, although they may be less immunogenic. They include fewer antigens, and the purifying process frequently removes components that elicit innate immunity. Subunit vaccinations include tetanus toxoid, inactivated seasonal influenza, acellular pertussis, and pneumococcal polysaccharide vaccines (Rappuoli et *al*., 2014), as well as anti-COVID-19 mRNA. vaccine (Cao et *al*., 2022). Subunit vaccines boost the immune system by targeting only a fraction of the pathogens. The system's reaction. This can be accomplished by isolating a particular protein from the pathogen. and delivering it individually as an antigen (World Health Organization, 2010; World Health Organization, 2016).

I.2.4. Recombinant vaccines

are manufactured through genetic engineering to incorporate a specific component of the pathogen's genetic material. (Islam & Rahman, 2023). The commonly used hepatitis B vaccine is an example of a recombinant protein vaccine (Michel & Tiollais, 2010) whereas the Human Papillomavirus (HPV) vaccine is manufactured through genetic engineering (Dai et *al*., 2019).

I.2.5. Toxic vaccines

Toxins produced by bacteria can be inactivated by heat or chemical treatments (e.g., formaldehyde), keeping their physicochemical features, structure, and immunogenicity (Blin, 2021). Vaccines target disease-causing toxins rather than the pathogen (Ghattas et *al*., 2021; Su et *al*., 2019). Examples include diphtheria and tetanus toxoid

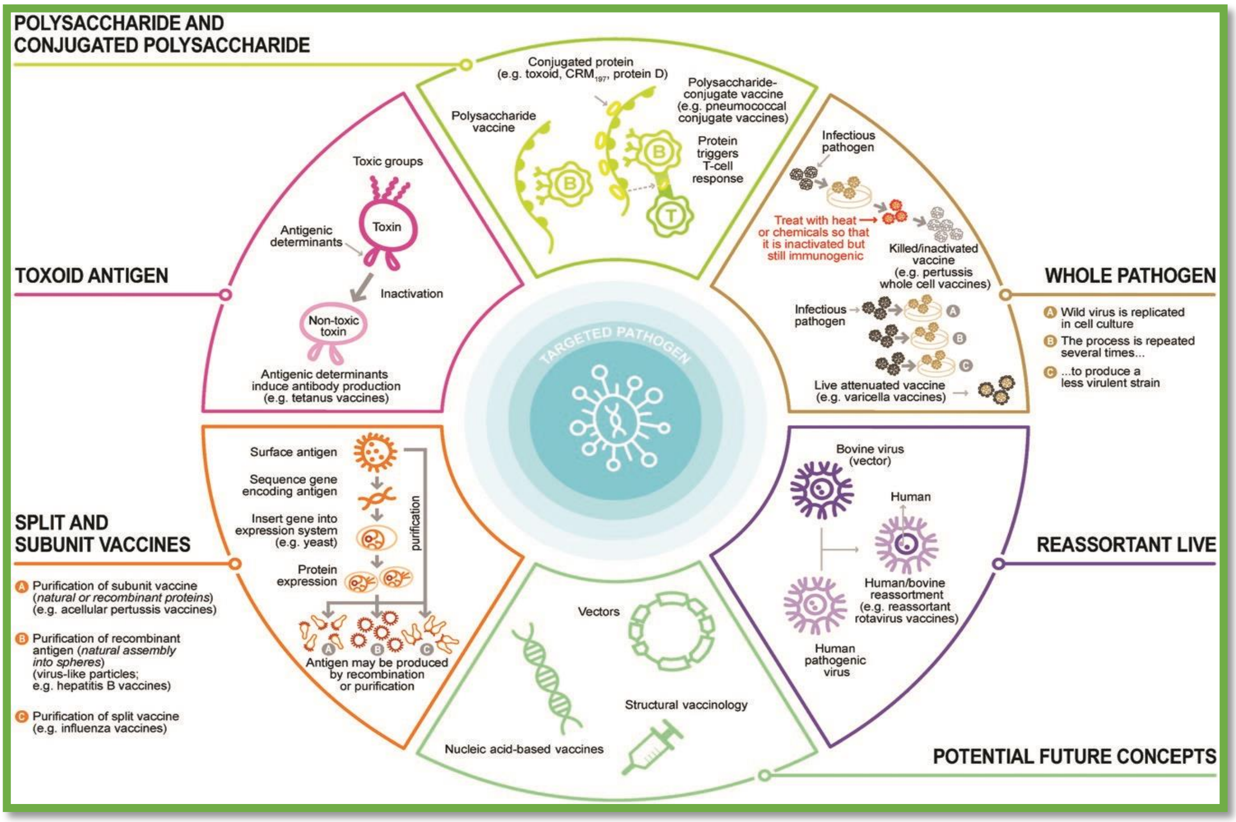


Figure 1: Different types of vaccines (Vetter et al., 2018).

I. 3. Administration of vaccines

Appropriate vaccine delivery is essential for effective immunization (Kozak & Hu, 2023). The most basic and widely used mode of delivery is subcutaneous (SC) or intramuscular (IM) vaccination. This method is obviously ideal for small numbers of animals and illnesses requiring systemic immunity (Tizard, 2020).

I.3.1. Intramuscular (I.M.) routes

Intramuscular delivery improves the immunogenicity of most immunizations while reducing adverse responses at the injection site. The muscles are highly vascularized, allowing the vaccine to be easily mobilized and transported throughout the body's lymph tissue (Zuckerman, 2000).

I.3.2. Subcutaneous (S.C.) routes

Subcutaneous injections are delivered into the fatty tissue beneath the dermis (CDC, 2023). Subcutaneous injection is linked with much lower seroconversion rates and a faster decay of antibody response than intramuscular injection. However, with certain vaccinations, particularly live-attenuated vaccines, this is generally beneficial to limit morbidity since the antigen may take longer to enter the circulation after being deposited in fat, which has fewer blood vessels than muscle. This reduced vascularity, similar to a depot, causes a delayed and more persistent release, delaying processing by macrophages and eventual presentation to T and B cells implicated in the adaptive immune response (Zuckerman, 2000; Geddes, 2021).

I.3.3. Intradermal (ID) routes

It offers a prospective alternative to I.M. or S.C. immunizations, such as the Bacille Calmette-Guérin (BCG) vaccine for TB, lower dosage rabies vaccines, and DNA vaccines (Kim et *al*., 2012). Dendritic cells in the epidermis stimulate mucosal and systemic immunity (Peletta et *al*., 2023). Intradermal immunization uses novel needle-free technologies, including high-pressure stream injectors and electroporation. (Ahmed et *al*., 2023)

I.3.4. Oral vaccination

Oral vaccines have been proposed as a potential vaccination against a variety of infections, particularly those that invade the gastrointestinal system. One important aspect of oral vaccines is the effective transport of antigen to gut-associated lymphoid tissue (GALT) (Kunisawa et al., 2012). There are significant benefits to successful vaccinations, particularly for immunization against enteric infections since the gut mucosa contains immune tissues. Three presently licensed oral vaccinations protect against intestinal pathogens: the oral poliovirus (Sabin), the cholera vaccine, and the rotavirus vaccine (droplets in the mouth). The primary benefits of oral vaccination are logistical in nature, since they are generally easier to make and transport, making them significantly less expensive than injectable vaccinations. They are also more stable, having longer shelf lives, making global distribution easier. Furthermore, many people, including trypanophobes, are afraid of needles. These vaccinations are straightforward to administer and need little training; they are also more readily dispersed than solutions (Kozak & Hu, 2023).

I.3.5. Intranasal vaccination

It is well known that when antigens enter the human body, they have their first contact with the nasal cavity. As a result, the development of nasal vaccines may be a viable option for more effective vaccination (Bahamondez-Canas& Cui, 2018; Jabbal-Gil, 2010). Many research studies have been conducted to demonstrate the efficacy of intranasal (IN) vaccination, with some undergoing clinical trials. These vaccinations may be used to treat both respiratory and systemic illnesses. The nasal cavity is rich in lymphatic tissue, often known as nasal-associated lymphoid tissue (NALT), which combines humoral and cellular immune responses, producing both systemic and mucosal immunity. In addition, immunoglobulin A (IgA), which accounts for more than 15% of total immunoglobulins, is found in greater concentrations in mucosal secretions than in serum The dominance of IgA in the nasal mucosa plays a vital role in protecting the mucosal surface by inhibiting the attachment and/or penetration of antigens through the nasal epithelium (Yusuf & Kett, 2017). Research findings (Lijek et al., 2012; Jang et al., 2012) have demonstrated that stimulating IgA production, alongside serum IgG, enhances the efficiency of intranasal (IN) immunization by generating cross-reactive antibodies. This cross-reactivity allows for reduced vaccination frequency, as these antibodies can respond to multiple antigens (Yusuf & Kett, 2017).

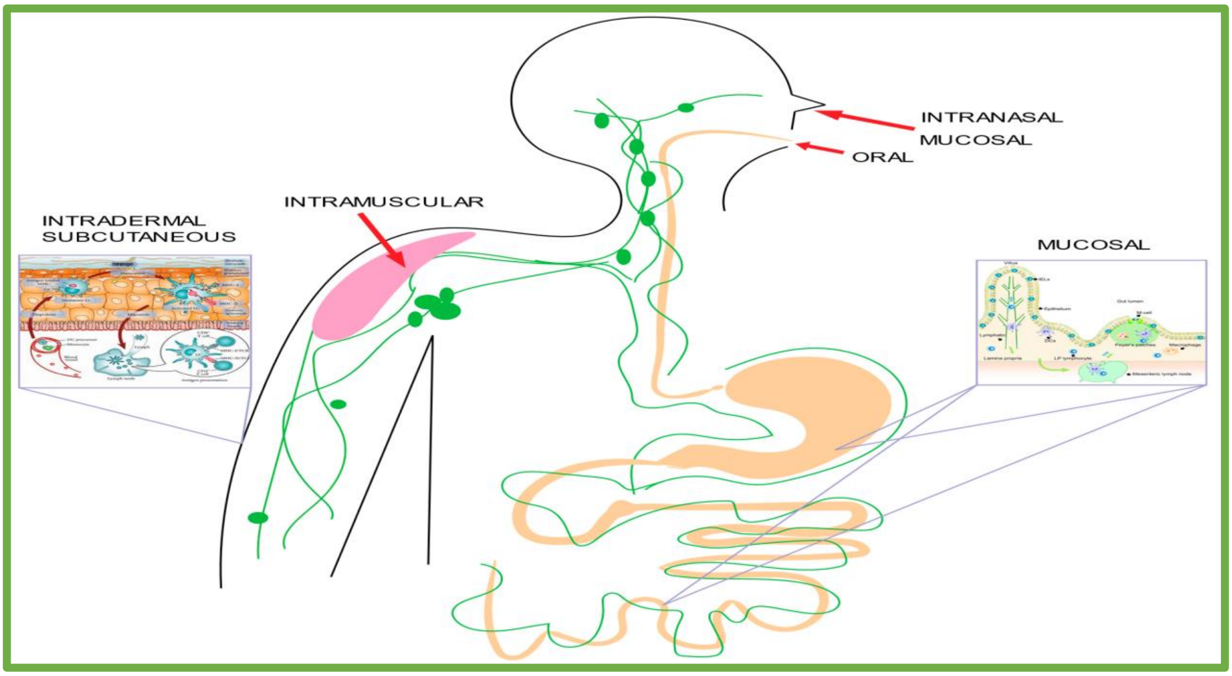


Figure 2: Vaccine Delivery Routes (Peiser, 2013; Wu et al., 2014)

I.4. Mechanism of action of a vaccine

Vaccines are intended to imitate infections and stimulate individual immunity to illness (Yadav et *al*., 2018). The innate immune system is the primary defense against foreign chemicals, reacting in hours with little specificity or memory (Vetter et *al*., 2018). Vaccination is intended to protect against infectious illnesses by stimulating immune responses to non-pathogenic microbial forms or components. The vaccine's active component, an immunogen, induces a protective response that includes both innate and adaptive immunity (Miot et *al*., 2019).

There are two sorts of reactions: primary responses, which occur immediately after the first injection, and secondary responses, which are triggered by the second injection, or booster, which happens more than a month later. The major response is a complicated interaction of innate and adaptive immunity (Autran et *al*., 2016; Zepp, 2016; Moser & Leo, 2010). Antigen-presenting cells (APCs) deliver the antigenic preparation to CD4+ T cells, initiating the vaccination response. The vaccine formulation affects this first phase as well as the quality of the immune response. Vaccine antigens must mimic the natural pathogen's first signal, which is recognized by innate immune cells' pattern recognition receptors (PRRs). Certain vaccine antigens bind to APCs using toll-like receptors (TLRs) (Barton & Medzhitov, 2002). Activating an inflammatory cascade that results in APC migration to the draining lymph node and cytokine production. This activates other immune cells, including naïve CD4+ T cells, by presenting antigenic peptides to MHC-II molecules. B cells, which also function as APCs, get activated when exposed to the vaccination antigen and develop into plasmocytes that produce low-affinity IgM antibodies. B cells are activated in the presence of CD4+ T cells, causing them to create high-affinity IgG or IgA antibodies as well as memory cells via genetic changes. This emphasizes the significance of activating CD4+ T cells and APCs, particularly dendritic cells, and the use of adjuvants (Zepp, 2016).

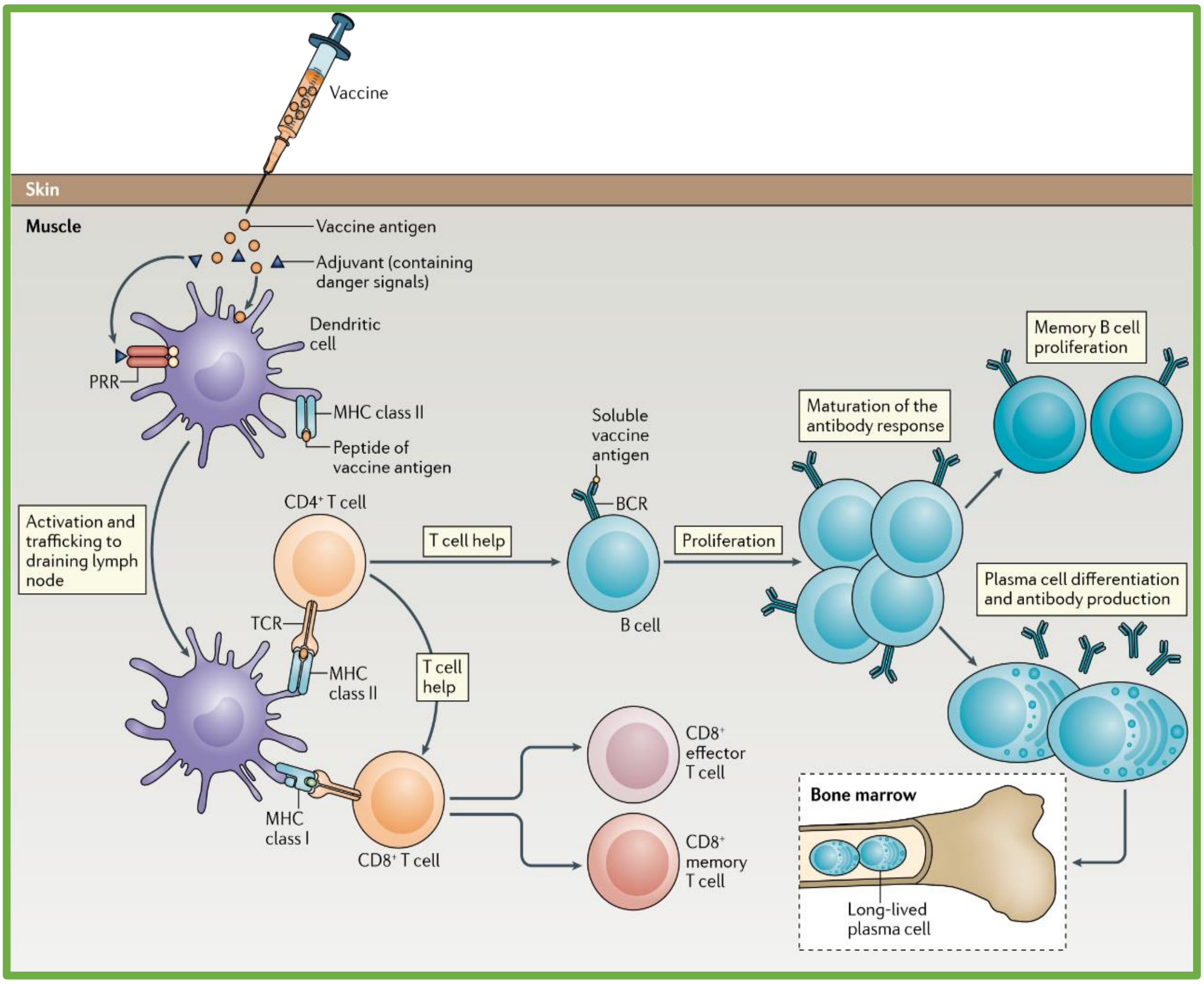


Figure 3: The generation of an immune response to a vaccine (Pollard & Bijker, 2021)

I.5. Side-effects of vaccination

Vaccine recipients can primarily expect the following symptoms during the early phase of the post-vaccination period: localized soreness, generalized weakness, myalgia, headache, chills, fever, joint pain, and nausea. Muscle stiffness or spasm, sweating, dizziness, flushing, feelings of joy/relief/gratitude, brain fogging or reduced mental clarity/attention/concentration, decreased appetite, localized swelling at the injection site, decreased sleep quality, itching, tingling, diarrhea, nasal stuffiness, and palpitations and /or high heart rate were reported as other predominant symptoms (Kadali et *al*., 2021). Common local reactions to vaccines include pain, swelling, and erythema at the injection site. Systemic reactions, including fever, irritability, drowsiness, and rash, may also occur (Spencer et *al*., 2017).

Chapter II

Scorpion Venom

II.1. Scorpion venom

Scorpion venom is a natural biological resource that comprises numerous components that not only cause death but may possibly have medicinal properties. Traditional and folk medicine utilizes scorpion venom to treat a variety of pathological diseases (Akef, 2019). Over 1700 species have been described so far (Stockmann et *al*., 2010) .Scorpions are categorized into 18 different families (Prendini L, 2005) .30 genera of the Buthidae family present human threat (Caliskan F et *al*., 2013)

II.2. Chemical components scorpion venom

Scorpion venom is a complex and diverse collection of bioactive chemicals, including peptides, enzymes, mucoproteins, free amino acids, nucleotides, lipids, amines, and heterocyclic compounds, as well as inorganic salts. The molecular variety of scorpion venom is a sign of its complexity, with each component having a distinct function in the venom's overall biological activity and therapeutic potential (Ortiz et *al*., 2015). Scorpions can generate neurotoxic, cardiotoxic, and hemolytic toxins, producing mild to moderate symptoms such as numbness, neuralgic or stabbing pain, general discomfort, disorientation, irritability, and headaches (Ochoa-Andrade et *al*., 2022). Nonetheless, venom has emerged as a potent instrument for addressing many issues and developing solutions in a wide range of fields, including pharmacology and medicine (Vega et *al*., 2015)

II.3. Symptoms caused by scorpion venom

The frequency of scorpion envenomation complaints grows year after year. The uncontrolled spread of dangerous scorpion species, as well as the growth of metropolitan areas, contributes to an increase in scorpion encounters (Ahmadi et *al*., 2020; Ward et *al*., 2018). Every year, around 1 million scorpion envenomations occur, resulting in nearly 3250 fatalities (Cid-Uribe et *al*., 2020). It was recently revealed that scorpion stings cause a range of symptoms, the most serious of which are cardio-respiratory dysfunctions (Sifi et *al*., 2020), bleeding (Shah et *al*.,2018), and hematological (disseminated intravascular coagulation [DIC]), renal (acute kidney damage), and neurological (seizures, autonomic dysfunction) Ischemic or hemorrhagic stroke (Cupo, 2015; Abroug et *al*., 2020) and even local tissue necrosis (Jenkins et *al*., 2018). It is believed that the intensity and systematicity of scorpion envenomation are directly connected to venom neurotoxicity. effects, namely neuronal stimulation and catecholamine release (Al-Asmari et *al*., 2016; Isbister et *al*., 2014; Ortiz et *al*., 2015). However, the development of severe systemic symptoms may be connected with increased enzymatic activity inside the tissues, which triggers the inflammatory response. This also triggers the inflammatory response (Minutti-Zanella et *al*., 2021; Petricevich, 2010).

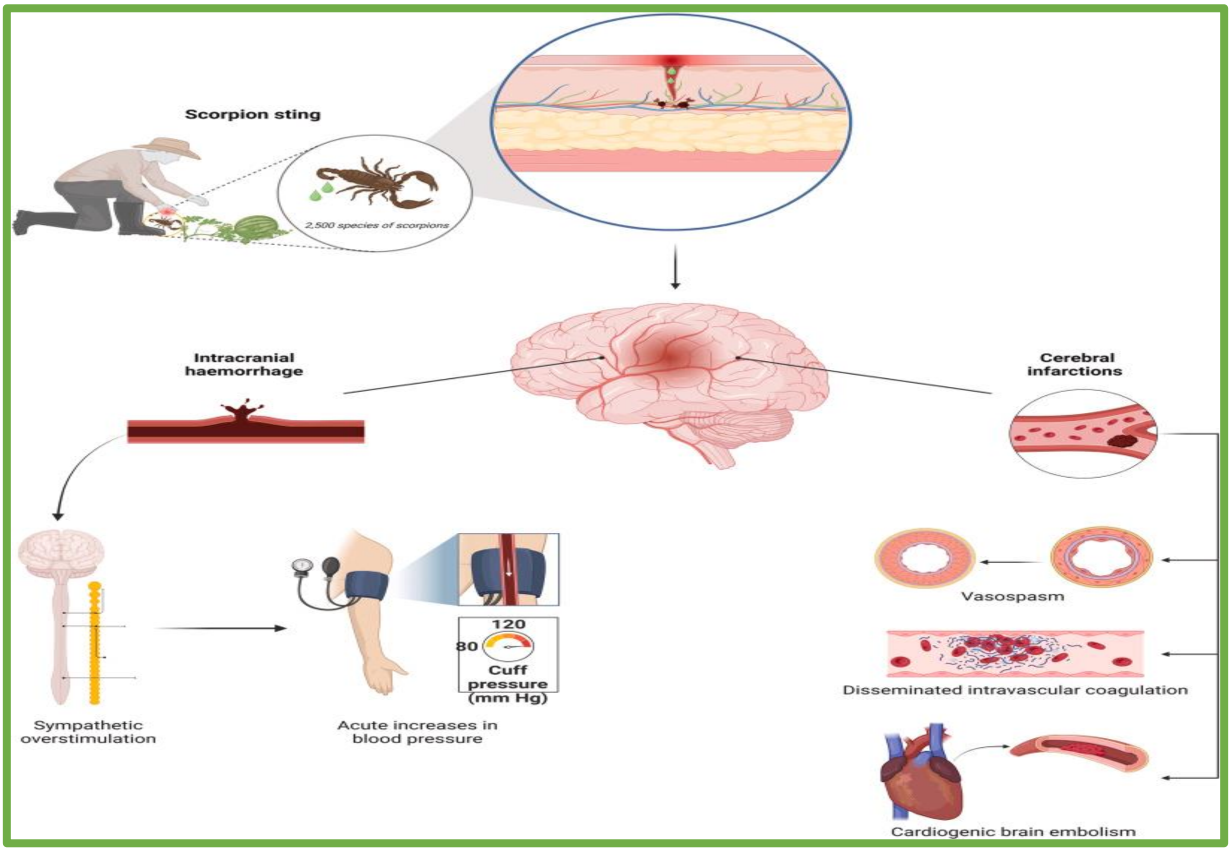


Figure 4: Symptoms caused by scorpion venom (Gunas et al., 2023)

II.4. Scorpion venom extraction

- **Venom extraction with electricity**

Another approach for venom extraction was electrical stimulation of the telson. The approach given by (Yaqoob et *al*., 2016) was used. The scorpion was taped to a petri plate to remove its venom. Electric current (25 V) (Yaqoob et *al*., 2016) was administered to the base of the telson for 5 seconds using a pointed electrode to shock the scorpions until the venom was released (Fig. B). To get greater conductivity of electric current, we submerged the scorpion body in a 10% NaCl solution. The venom was collected in a graduated capillary tube and diluted with distilled water at a 1:5 ratio. Further usage. Diluted venom was spun at 14,000 rpm for 10 minutes in a centrifuge (MPW-352R, Germany). The supernatant was kept at -20°C for future use.

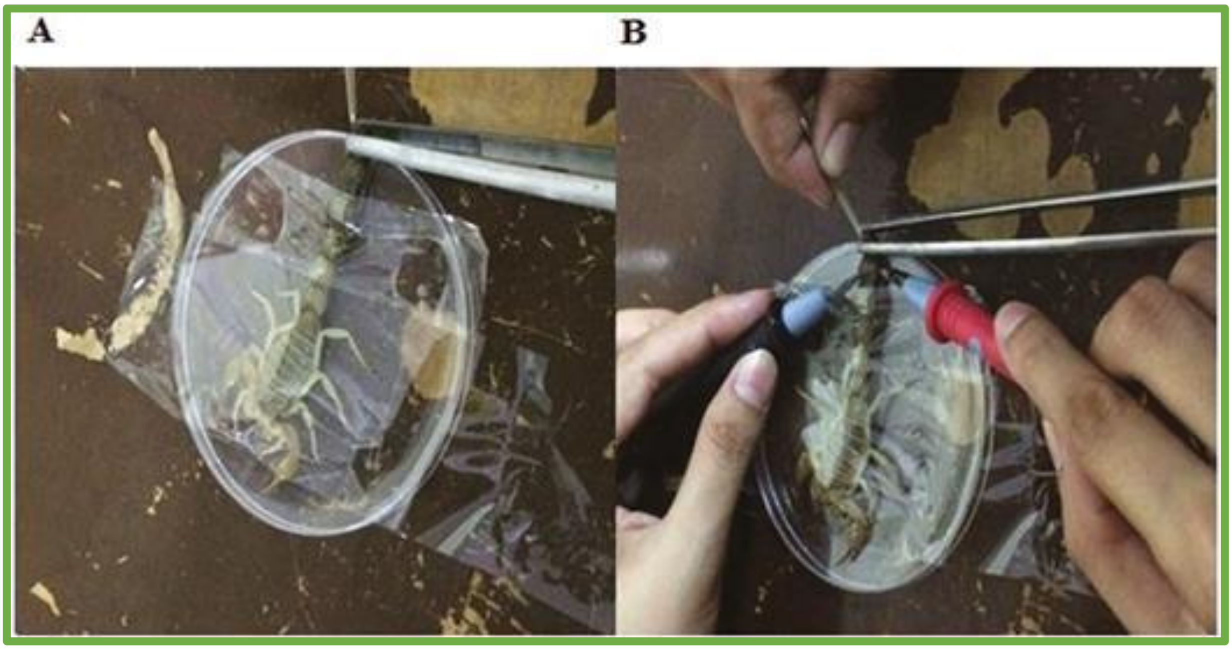


Figure 5: Methods of venom milking in scorpions: (A) manual method and (B) electrical method (Tobassum et al., 2018)

- **Yearly Extraction of Venom**

Louis's (1976) hand-milking technique was used to extract A. finitimus and H. tamulus venom. Scorpion was put on a petri plate using tape in this technique; their stinger was inserted into the point of the graded capillary tube (Fig. A). Then scorpions' abdomens were physically agitated, causing them to exude poison. Indeed, several researchers demonstrated that manually milked venom is the first form of venom, known as prevenom, whereas venom extracted electrically corresponds to the second type (Oukkach et *al*., 2013).

II.5. Benefits of scorpion venom

Current Research on Scorpion Toxins with Potential Therapeutic Applications. It is well stated in the literature that scorpion venom has a high concentration of bioactive chemicals, making its toxins appealing to the pharmaceutical and biotech sectors (Kerkis et *al*., 2017). However, despite the fact that much research is underway and the possibilities for scorpion-derived therapeutic peptides are highly intriguing, chlorotoxin is the only toxin from scorpion venom that has been tested in clinical trials (ClinicalTrials.gov, 2019). Furthermore, no scorpion toxin-based drugs are currently available in the market (Pennington, et *al*., 2018).

The therapeutic potential of many biochemical substances found in scorpion venom has promise in a variety of sectors, including biotechnology, cancer therapy, and treatment of neurodegenerative and cardiovascular diseases (Kazemi & Sabatier, 2019).

II.6. The potential therapeutic applications of scorpion venom

El veneno de diversas especies de escorpión encuentra importantes usos terapéuticos. Como la potenciación de bradykinin, la hemólisis, la anticancerígena, la antimicrobiana y la antiinflamatoria, son reguladas por péptidos no disulfide-bonded. Por lo tanto, es estimulante utilizar estas propiedades en el tratamiento de cáncer, enfermedades cardiovasculares, diabetes, AIDS, apoplexy, influenza H5N1, paralysis, epilepsy, malaria, measles, etc (JAVED et *al*., 2022)

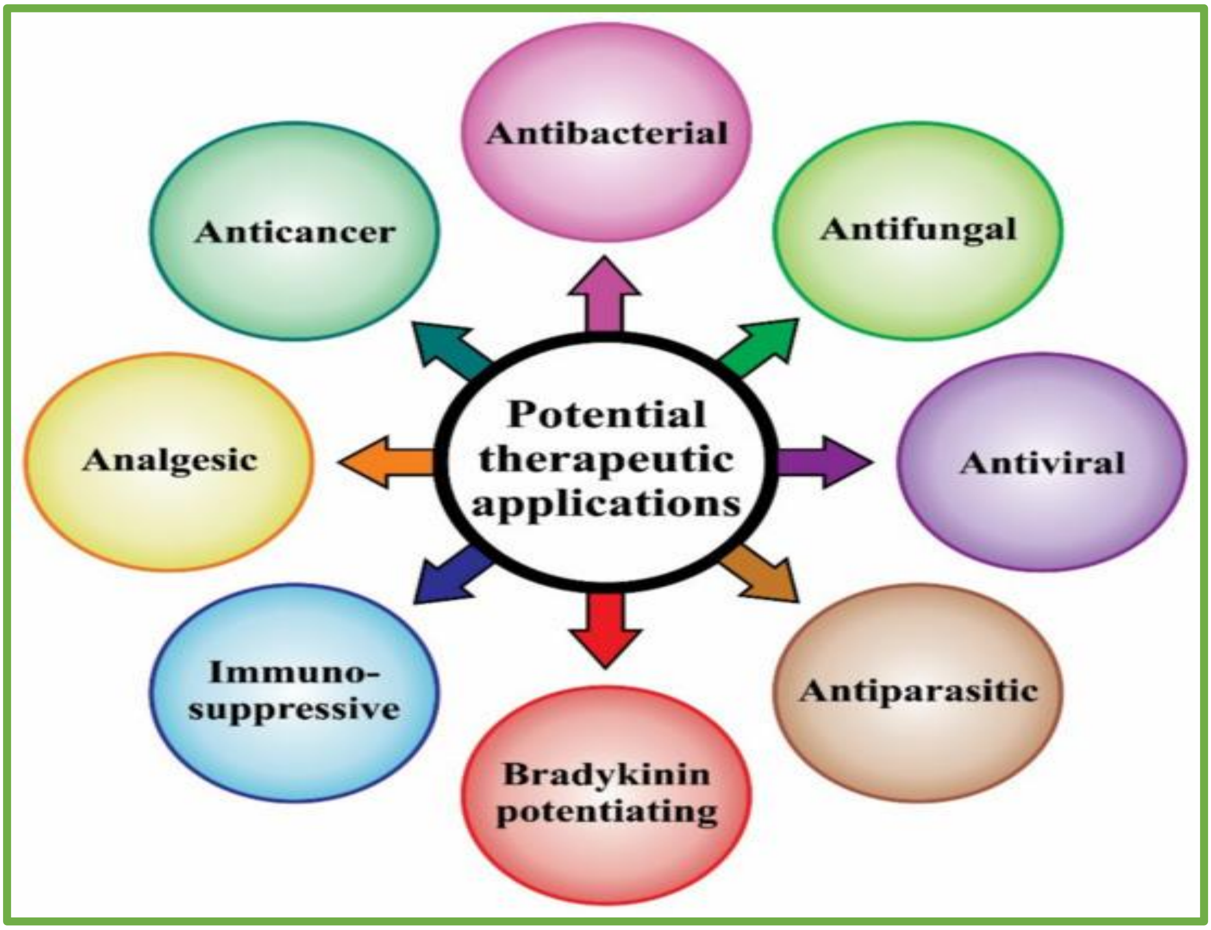


Figure 6: The potential therapeutic applications of scorpion venom (Ahmadi et al., 2020)

II.7. Harmful effects of scorpion venom

Scorpion envenomation can produce serious clinical consequences and even death in people. The degree of an envenoming is typically determined by the victim's sensitivity and body mass, the anatomical position of the sting, the volume of venom delivered, and the type of scorpion. Scorpion envenomings are often divided into three stages based on the severity of the symptoms (Cupo, 2015; Pucca et *al*., 2015). Mild envenomings cause local inflammatory reactions, but moderate and severe envenomings can lead to fatal systemic responses.

Chapter III

Nanoparticles

III.1. Nanotechnology

Nanotechnology is one of the most promising 21st-century technologies. Nanotechnology involves seeing, measuring, manipulating, assembling, controlling, and manufacturing materials at the nanoscale scale (1-100 nm) (Bayda et *al*., 2019). Nanotechnology is the science and engineering of designing, synthesizing, characterizing, and applying materials and devices with functional organization on the nanometer scale (Saini et *al*., 2010).

Rapid advancements in science and technology have led to new prospects for nanotechnology in medicine, electronics, food, and the environment (Morais et *al*., 2014). Nanotechnology is a rapidly growing field with several applications across various fields (Gatoo et *al*., 2014). It integrates information from physics, chemistry, biology, materials science, health sciences, and engineering (Godwin et *al*., 2015; Linhai et *al*., 2018) suggest that bio-nanotechnology is a cost-effective and environmentally benign method for producing nanoparticles and nanomaterials. Nanotechnology is increasingly being applied in medicine to prevent and treat illnesses (Gopal et *al*., 2010).

III.2. Nanoparticles

Nanoparticles (NPs) are unique structures that range in size from 1 to 100 nm. The category of NPs includes categories including organic, Inorganic and carbon-based nanoparticles, depending on their origin, characteristics, form, and dimensions (Prajapati et *al*., 2024). The broad mixing of nanoparticles throughout areas such as electrical gadgets, cosmetics, as well as both medicinal and analytical clinical uses, may be attributed to their small size being balanced by a large area. (Missaou et *al*., 2018) NPs serve as carriers for medications, facilitating both healing and analysis/diagnosis functions. Strong nanoparticles, liposomes, polymeric NPs, and nanoemulsions are among the types approved for use in medicine. There are several aspects affecting their physical properties, including chemical properties, drug loading efficacy, drug release, and, importantly, the carrier's non-toxicity, which determines its feasibility for healing applications. (Puri et *al*., 2009).

III.3. Types nanoparticles

III.3.1. Organic nanoparticles

In general, nanoparticles are divided into two categories: organic and inorganic Organic nanoparticles (eg, micelles, dendrimers, ferritin, and liposomes) are nontoxic and biodegradable. It is also worth noting that nanocapsules with hollow cores, such as micelles and liposomes, are sensitive to thermal or electromagnetic (EM) radiations such as heat and light.

These remarkable characteristics make them ideal for biological applications, particularly drug delivery (Salel & Iyisan, 2023). Nonetheless, (Naseri et *al*., 2015) note that poor stability, a short shelf life, and limited drug encapsulation performance may limit their broad use in drug delivery applications. A comparison of the characteristics of organic and inorganic nanoparticles (Poon & Patel, 2020).

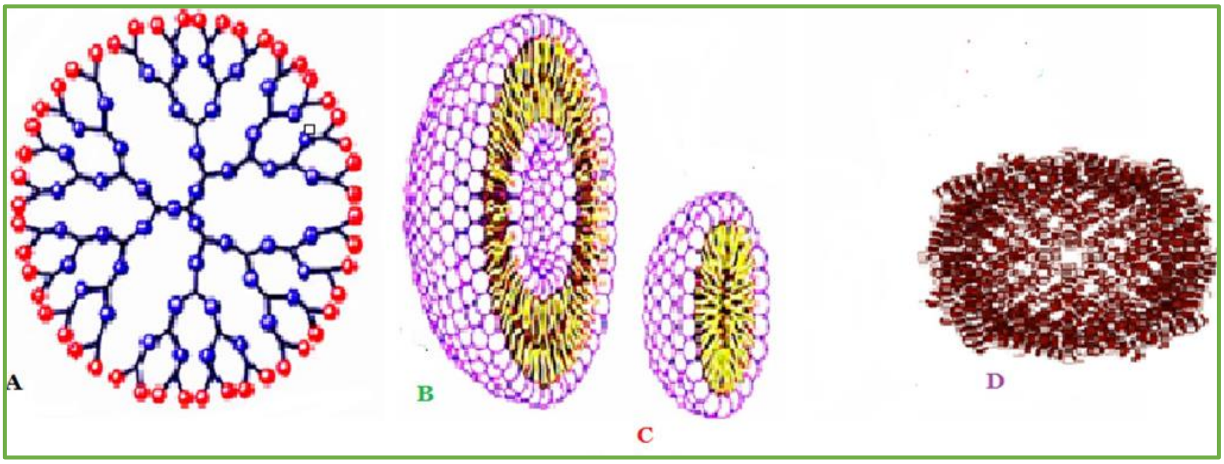


Figure 7: Types of organic nanoparticles (Zhang et al., 2016 ; Ijaz et al., 2020).

A, dendrimers ; **B**, liposomes ; **C**, micelles ; **D**, ferritin

III.3.2. Inorganic nanoparticles

Inorganic nanoparticles are more stable and hydrophilic than organic nanomaterials (Paul W ,2020). Inorganic nanoparticles do have intrinsic exceptional physicochemical features (magnetic, thermal, optical, and catalytic performance), and consequently, these nanosized materials offer a robust framework where two or more dopants may be merged to supply m Zhang X ultifunctional abilities (Liong et *al*., 2008; Zhou H et *al*., 2020; Zhang et *al*., 2012; Zhang X et *al*., 2012). Inorganic nanoparticles outperform organic nanoparticles in terms of drug loading capacity, stability, and adjustable degradation rates (Mishra P et *al*.,2022; Oh JY et *al*., 2022; YuY et *al*., 2022).

III.4. Applications of nanoparticles

- **Medicine precision delivery**

This decreases medication use, minimizes side effects, and improves healing outcomes. Nanotechnology's role in cell design offers alternatives to traditional therapies like artificial implants and organ transplants. Examples include using carbon nanotube structures for bone formation and incorporating gold into Ayurvedic treatments (Ganesh et *al*., 2013; Mudshinge et *al*., 2011)

- ❖ **Diagnostics imaging agents**

Nanoparticles (NPs) help see specific bodily areas during diagnostic procedures (Ganesh et *al*., 2013; Mudshinge et *al*., 2011)

- ❖ **Tissue engineering stimulators**

NPs contribute to cell design by encouraging the formation and repair of cells and bodily organs. (Siddique & Chow, 2020)

- ❖ **Cancer therapy enhancements**

Nanoparticles improve cancer cell therapy by delivering directly to growth areas. Targeted pharmaceutical distribution reduces damage to healthy and balanced cells, increasing therapeutic efficacy while lowering side effects (Ganesh et *al*., 2013)

- ❖ **Neurological disorder treatments**

NPs effectively treat neurological diseases by facilitating medicine transport across the blood-brain barrier. Their small size and unique features make them excellent carriers for drugs targeting the central nervous system, perhaps leading to treatments for diseases such as Alzheimer's and Parkinson's. (Ganesh et *al*., 2013)

- ❖ **Gene therapy advancements**

Nanoparticles play a crucial role in genetic treatments by delivering hereditary products to specific cells. This strategy is effective in treating genetic disorders and other illnesses with personalized medicine. (Ganesh et *al*., 2013).

III.5. Copper oxide nanoparticules (CuO NPs)

Copper (Cu) is one of the eight necessary micronutrients and plays an important role in the formation of metalloproteins, acting as a cofactor in the control of enzyme activity(Kaur & Srivastava, 2023). It has a critical function in controlling the combination of diverse macromolecules that have cardinal metabolic activities in plants, including photosynthesis, respiration, lignification of cell walls, and several defense mechanisms against abiotic stressors (Lodde et *al* .,2021) .Copper oxide nanoparticles (CuO NPs) have lately sparked widespread attention due to their unique optical, electrical, magnetic, biological, and catalytic capabilities (Jeronsia, *et al*.,2019; Dastjerdi & Montazer ,2010; Mohmmed *et al*.,2021). Copper nanoparticles (CuNPs) are gaining popularity due to their unique physical and chemical characteristics, as well as ease of manufacturing (Derouiche et *al*., 2022). These intriguing properties have stimulated substantial research into CuO NPs in a variety of sectors, including energy, electronics, cosmetics, biosensors, storage devices, supercapacitors, catalysis, food and agriculture, and healthcare (Fig. 1) (Katwal, *et al*., 2015; Aaga & Anshebo ,2023). Furthermore, their antibacterial and anticancer characteristics have established them as prospective therapeutic agents (Qamar, *et al*., 2020; Rehana, *et al*., 2017; Singh, *et* al., 2023). With the growing need for ecologically friendly and sustainable synthesis methods, biogenic synthesis (green synthesis) has gained prominence in the production of CuO NPs. This method uses biological entities, such as plants and microbes, to manufacture CuO NPs, which has benefits in terms of availability, simplicity, cost-effectiveness, and environmental compatibility (Akintelu & Folorunso, 2019; Subbaiya & Selvam, 2014). Green synthesis eliminates the use of hazardous chemicals by employing natural reducing, capping, and stabilizing agents found in biological sources, which prevents nanoparticle agglomeration (Letchumanan et *al*., 2021;Narayanan & Sakthivel, 2010;Sharma et *al*., 2015) Recent studies have emphasized the potential applications of CuO NPs synthesized using green methods, such as antimicrobial (Almasi *et al*., 2018 ; Rajesh *et al*., 2018), anticancer agents (Gnanavel *et al*.,2017 ; Saravanakumar et *al.,* 2019);Sarfraz *et al*., 2019 ;Sarfraz, *et al*., 2023*)*, antioxidants (Rehana *et al*., 2017), drug delivery vehicles (Mariadoss *et al* ., 2020;Mohammadhassan *et al.*, 2022 ; Assadi *et al*., 2018), anti-inflammatory agents (Velsankar *et al*., 2020*)*, antidiabetic agents (Faisal *et al*., 2022*)*,and antitumor agents (Maor *et al*., 2021; Dey *et al*., 2020;Benguigui, et *al.*, 2019).

These nanoparticles have exceptional antibacterial activity, making them useful as wound dressings and antiseptics (Sankar *et al*., 2015*)*. They also show fungicidal activity against a variety of fungal strains (Rabiee *et al.*, 2020; Devipriya& Roopan et *al*., 2017). In addition, CuO NPs function as excellent nonenzymatic biosensors for therapeutically important analytes and show potential as nanocarriers and antitumor agents in cancer therapy (Xu et *al*., 2018; Reddy et *al*., 2012; Uzunoglu & Stanciu, 2016; Chen et *al*., 2016; Naz et *al*.,2018).

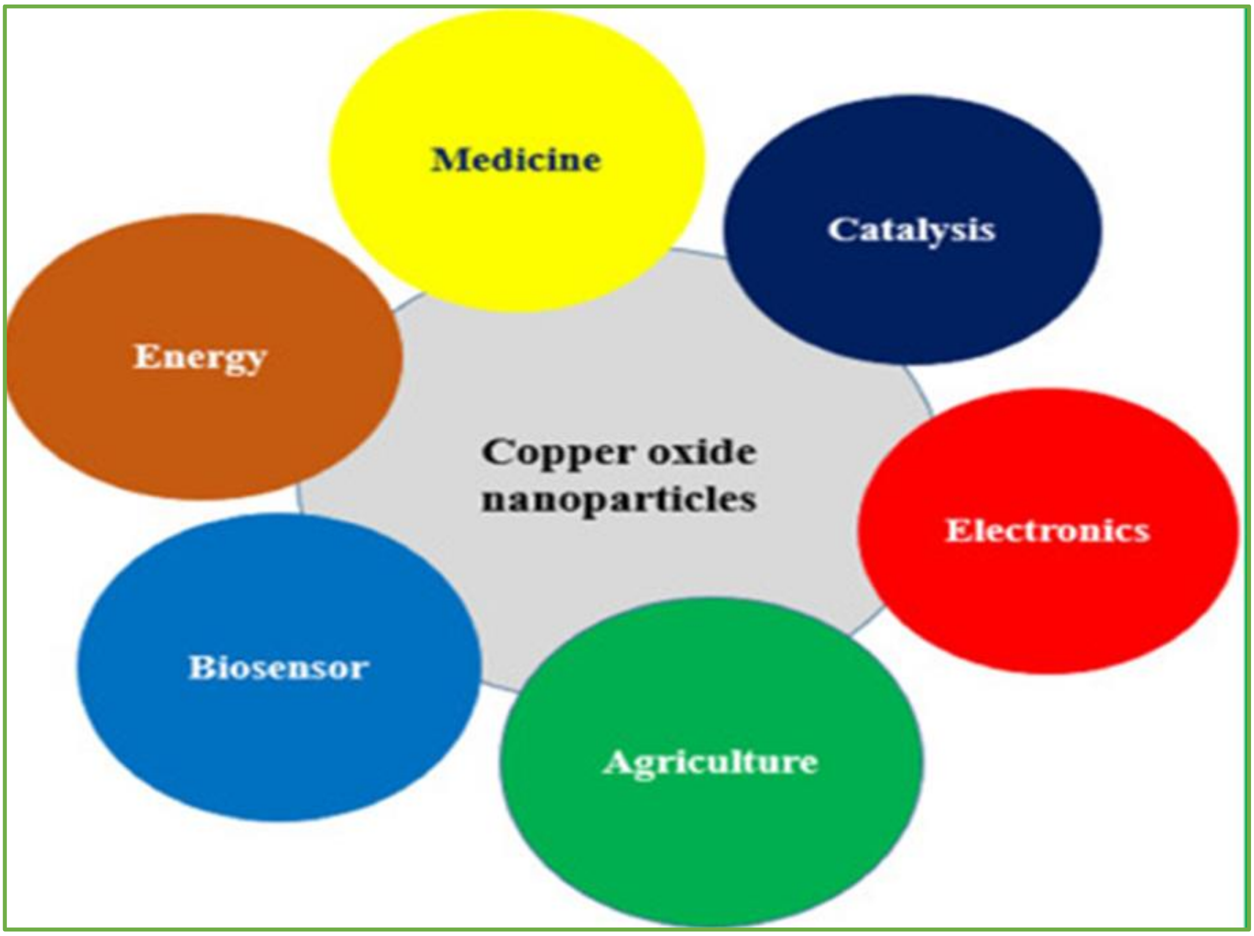


Figure 8: General applications of CuO NPs (Gebreslassie & Gebremeskel, 2024)

III.6. The green synthesis

is a cost-effective, environmentally friendly, and simpler method of producing stable nanomaterials with specific size and shape. which uses bacterial, fungal, and plant (Singh et *al*., 2020) cell cultures and is influenced by temperature, pH, reaction time, and reagent volumes (BenMosbah et *al*., 2022; Jahan et *al*., 2021). Biosynthesis of nanoparticles employing natural reagents such as vitamins, sugars, plant extracts, biodegradable polymers, and microbes is a promising approach for nanotechnology (Derouiche et *al*., 2022). The technique has the potential to alter materials science by allowing the manufacture of nanoparticles with improved characteristics and lower environmental impact (Ding et *al*., 2014). This technology has various advantages over chemical and physical procedures, including non-toxicity (Devi et *al*., 2019), zero pollution (Alsammarraie et *al*., 2018), environmental sustainability (Kataria & Garg, 2018), cost-effectiveness, and more sustainability (Nasrollahzadeh & Mohammad Sajadi, 2016)

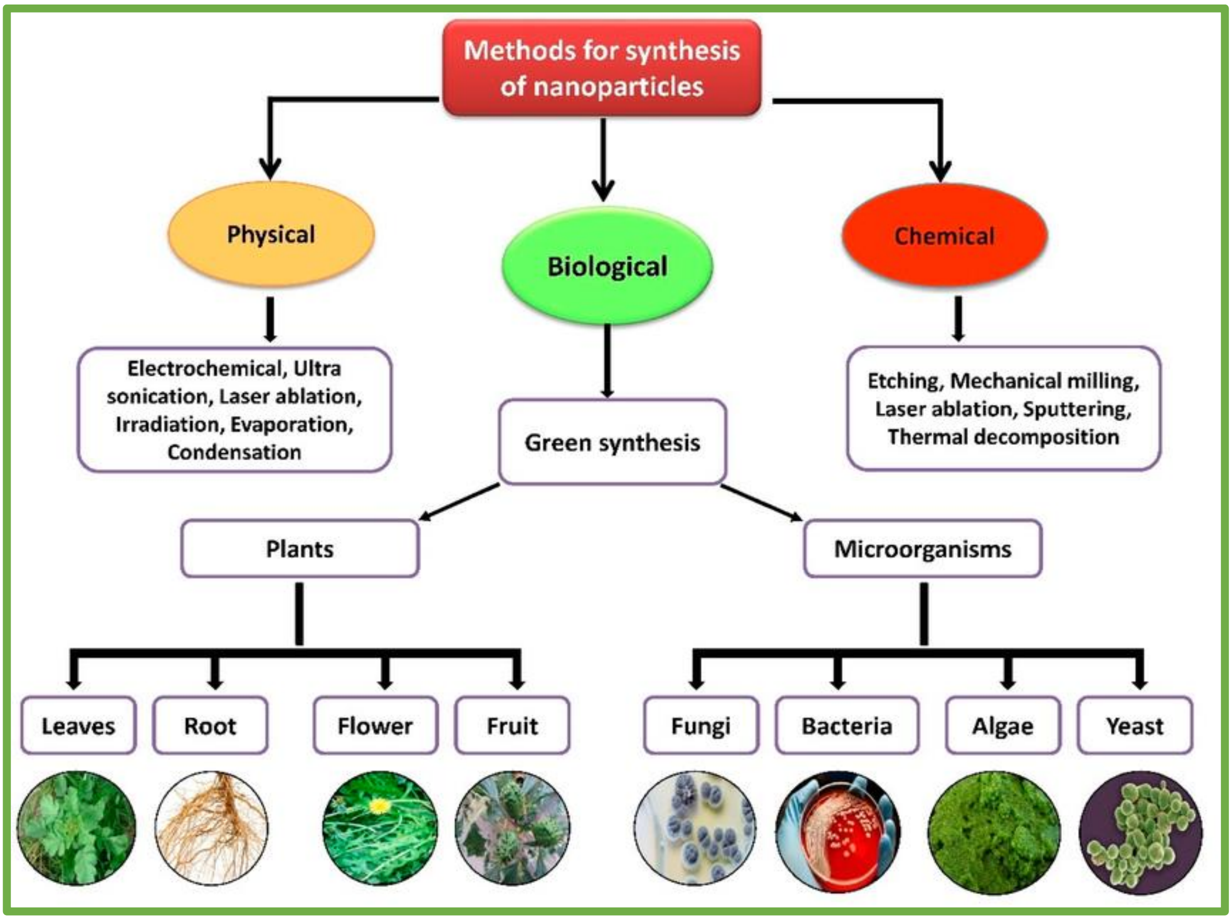


Figure 9: Methodes for synthesis of nanoparticles (Khan et al., 2022)

III.7. Toxicity of nanoparticles

Examines the negative impact of manmade nanomaterials or nanoparticles on living beings. The use of artificial nanoparticles for biological applications has sparked considerable concerns regarding their safety. humans. Due to their tiny size, nanoparticles (NPs) are frequently employed in nanomedicine and as drug carriers. and unique characteristics (Farah, 2019; Zhu et *al*., 2019). However, their size is (Chen et *al*., 2019). Morphology, surface functional groups (Renero et *al*., 2019), and dose-dependent characteristics (Cheicherd et *al*., 2019) may also be responsible for them. poisonous to normal, healthy human cells, tissues, and the organs. The toxicity of some secondary particles has been demonstrated to vary on both size and specific surface area (SSA) (Huang et *al*., 2017). Chemically synthesized nanoparticles are more harmful to human cells than biosynthesized nanoparticles with biocompatible surface functional groups, according to many studies (Jeevanandam et *al*., 2016). Biosynthesized nanoparticles can be harmful when they mix with cells, disintegrate into simpler forms, or accumulate (Naz et *al*., 2020; Roy et *al*., 2015).

Second part

Experimental Part

Chapter I

Materials & Methods

I.1. Materials

I.1.1. Reagents and products:

Copper(II) chloride (CuCl2), Hydrogen chloride (HCl), chloroform, sodium chloride (NaCl), ascorbic acid (C6H8O6), Coomassie blue, orthophosphoric acid (H3PO4) , Riboflavin, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Butylated Hydroxy Toluene (BHT), Nitroblue Tetrazolium (NBT), Dipotassium phosphate (K2HPO4), Potassium dihydrogen phosphate (KH_2PO4), Ethanol, Ethyle nediaminetetraacetic acid (EDTA), physiological solution (Na Cl 0.9%), and distilled water.

I.1.2. Sampling and Extraction of venoum scorpion:

The venum samples utilized are sourced from scorpions, raised in (Dibela), El-Oued, Algeria.This document represents a scorpion venom extraction scheme (According to Louis, 1976). (Oukkach et *al*., 2013).

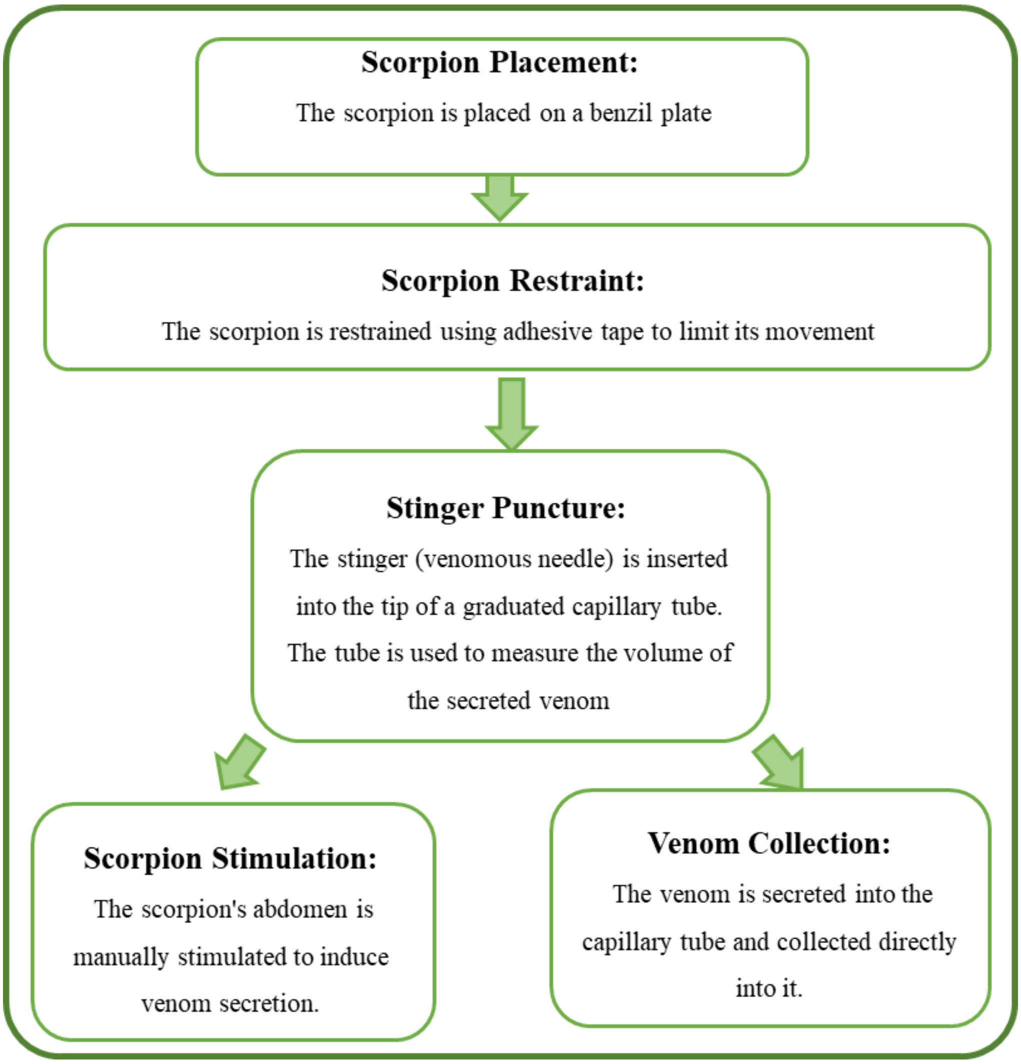


Figure 10: How to extract scorpion venom.

I.2.Animals

I.2.1. Animals care:

In this study, 8-week-old 15 male *Albino Wistar* rats weighing between 156-204 g were sourced from the Institute Pasteur of Algiers. They were housed in cages within the animal room of the Department of Molecular and Cellular Biology at the Faculty of Nature and Life Sciences, Echahid Hamma Lakhdar-El-Oued University, Algeria. The animals underwent a 7-day adaptation period under standard conditions of room temperature with a 12-hour light/dark cycle. Throughout the study, the rats were given free access to food and water.

I.2.2. Experimental design:

After a period of adaptation, the animals were divided into three groups (n = 5), as follows:

- **Group I (control group):** a non-vaccinated group injected intraperitoneally only with 0.5mL of physiological water.
- **Group II (non vaccinated group):** were injected intraperitoneally with 0.5 mL of physiological water. After two weeks, we were exposed intraperitoneally to 500 µl venoum scorpion (SV).
- **Group III (SV-CuNPs-vaccinated group):** were vaccinated by intraperitoneally injection (4 mg/kg) with SV-CuNPs synthesized by *Scorpion venoum*. After two weeks, we were exposed by injection to 500 µl venoum scorpion.

The CuNPs solution was obtained by ultrasonic technique for 60 min at 70 °C, and their body weights were recorded twice a week during the experiment period.

I.2.3. Sacrifice, blood sampling and tissue collection:

Four days after infection, the rats were sacrificed following a 16-hour fasting period. under slight anesthesia by chloroform (94%) by inhalation. During euthanasia, blood samples were collected from the animals and placed in EDTA tubes for hematological analysis and heparin tubes for biochemical analysis. The serum was obtained by centrifuging for 5 minutes at 2000 rpm, and blood sugar was measured by a glucometer. The weights of the organs were recorded, washed with saline (Nacl 0.9%), and frozen at -20 °C until use. The spleen, the heart, the kindey and the liver tissues are fixed in 10% formaldehyde for histological analoysis.

II.2. Methods

2.2. *In-vitro* study

2.2.1. Biosynthesis of Scropien venom by Copper Oxide CuO Nanoparticles

According to Khan et al.'s (2016) study, with minor modifications added. Extraction of starch from potato synthesis of copper nanoparticles using naturel starch as a capping agent and copper (II) sulfate pentahydrate as a precursor salt, the Cu nanoparticles were created via a chemical reduction method. The first step in the preparation process is to add 0.1 M copper (II) sulfate pentahydrate solution to 200 mL of 1.2% natural starch solution, stirring vigorously for 30 minutes. The synthesis solution is mixed continuously and quickly while 20 mL of a 0.2 M ascorbic acid solution is added in the second stage. The resulting solution was then heated to 80 °C for two hours while 30 mL of a 1 M sodium hydroxide solution was gradually added and constantly stirred. The solution changed from yellow to ocher in color. Following the reaction's completion, the mixture was removed from the heat source and let to settle for the entire night before the supernatant solution was carefully disposed of. To remove the excess starch bonded to the nanoparticles, the precipitates were filtered out of the solution and then three times rinsed with deionized water and ethanol.

- **Preparation of scorpion venom coated Copper-Nanoparticles (SV-CuNPs)**

venom solution (1µg/µL) was kept in a 30 mL tube with 2 mL of concentrated CuNPs to create immobilized venom. Using distilled water, the volume was increased to 10 mL, fully mixed, and rotated at a steady 500 rpm. The samples were centrifuged after four hours, and the resulting Copper-venom nanocomposite was then rinsed twice with distilled water and stored in a refrigerator at 4°C for additional research

2.2.2. Characterization of venom-Copper-Nanocomposites

The Cu nanoparticles synthesized using scorpion venom through a biological method were characterized using various techniques: the UV-Vis range of 250-600 nm, Fourier transform infrared spectroscopy (FTIR) to know the present functional groups associated with CuO in the range 400-4000 cm -1 and the morphological and elemental of NPs was determined using scanning electron microscopy (SEM) coupled with EDX. The zeta potential of synthesized SV-CuNPs powder in water was determined by Zeta sizer (NICOMPTM 380 ZLS). Transmission and scanning electron microscopy images were performed using TEM (TECNAI) An X-ray diffractometer (PROTO® AXRD Benchtop) was utilized to detect the chemical formula, crystallite structure, lattice parameters and crystallite size of CuO NPs. The

XRD was performed in the scaling angle (2θ) range of 10-80° with Cu Kα radiation (λ = 1.540593 Å). The crystallite size was determined using the Debye-Scherrer equation, which is written as D = k λ/β Cos θ. The Scherrer constant (0.9-1), the X ray wavelength (1.540593 Å), the full-width half maximum (FWHM), and the Bragg angle (θ), both stated in radians, are the parameters that are taken into consideration by the formula. Using formula $1/(d^2) =(h^2+k^2+l^2)/a^2$, where d is the interplanar spacing and h, k, and l are the Miller indices, the lattice parameters a, b, and c were found. XPS measurement on SV-CuNP surface was carried out using multiprobe photoelectron spectroscopy (Scienta Omicron) with Al K_α excitation of 1486.6 eV. During the measurement, sample surface was flooded with electron beam in order to avoid charging. The measured XPS peaks were analysed using CasaXPS software and calibrated with the reference C 1s value of 284.6 eV.

2.2.3. Disk Diffusion Assay

The Antibacterial activity was carried out using disc diffusion method (MWITARI et al., 2013). The disc diffusion assay on Mueller Hinton agar plates against common Gram-negative (Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922) and Gram-positive (Staphylococcus aureus ATCC 25923) bacteria was performed to evaluate the antibacterial properties of the synthesized SV-CuNPs. Bacteria were conserved on nutrient agar plates at 4°C. Activation of bacteria was performed at 37°C in the incubator, overnight. The 0.5 McFarland standard bacterial suspension was prepared in sterile physiological water. The suspension was spread uniformly on the dried surface of Muller Hinton agar by streaking swab three times. After that, sterile paper discs (Whatman No. 3) of 6 mm were impregnated with 20 µL (10, 20 and 30 mg/mL) of SV-CuNPs solutions, and discs were than dried in a clean bench before were placed on the inoculated agar surface. Discs impregnated with preparation solvents (sterile distilled water) were used as control. The all plate was incubated at 37°C for 24 h. Gentamicin and amoxicillin, were used as standards against all pathogens. After incubation, the zone of inhibition around each disc were measured in millimeters unit.

2.2.4 Determination of Protiens

- **Principle**

The tissue proteins were determined according to a colorimetric method by a SHIMATZU type spectrophotometer using Coomassie blue as a reagent, which is reacted with the amine group (NH2) of the proteins to form a blue complex. The appearance of the blue color reflects the degree of ionization of the acid medium and the intensity corresponds to the concentration of proteins. The absorption is measured at 595 nm (Bradford, 1976).

❖ **Preparation of Bradford's reagent**

- Dissolve 100 mg of Coomassie blue in 50 ml of ethanol (95%).
- Shake the mixture for 2 hours with a shaker away from light.
- Add 100 ml of orthophosphoric acid (H3PO4) (85%)
- Complete the volume to 1 liter with distilled water.
- Filter the solution obtained with filter paper.
- **Note**: This reagent is stable for 2 weeks at 4°C.

❖ **Procedure**

1. Take 01 ml of the protein solution.
2. Add 5 ml of Coomassie blue.
3. Shake and let to stand for 5 minutes.
4. Read the optical densities against the blank at 595 nm.
5. Compare the protein concentration in the tissues studied.

The protein concentration is determined by comparison with a standard range of bovine serum albumin (0.1-0.2-0.4-0.6-0.8-1mg / ml) previously carried out under the same conditions.

2.3.In-vivo study

2.3.1. Hematological parameter analysis

Hematological analysis by XN-330 (Sysmex). The metrics included the leucocytes, the erythrocytes, and the platelet line.

2.3.2. Biochemical and enzymatic parameters analysis

Urea, Creatinine, Cholesterol, Triglycerides, Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT) were measured using commercial kits (Spinreact).

2.3.3. Oxidative stress tests

2.3.3.1. Homogenate preparation

0.5 gram of tissues (liver, spleen, heart, and kidney) was grinded and homogenized in 5 ml of phosphate buffer solution (PBS, pH = 7.4). Homogenates were centrifuged at 5000 rpm for 15 min. The obtained supernatant was conserved at -20°C.

2.3.3.2. Determination of Malondialdehyde (MDA) level

❖ **Principle**

The common method for the assessment of MDA level is the thiobarbituric acid (TBA) assay, where MDA forms a complex with two molecules of 2-thiobarbituric acid (TBA) in the presence of acidic medium and heat. A change in the color of the solution to a pink color is an indication of the presence of MDA (Yagi, 1976).

❖ **Reagent**

375 mg of TBA, 20 g of TCA, 0.01 g of BHT, 25 ml of 1N HCL, and 50 ml of distilled water were mixed in a beaker. The solution obtained was heated to 40 °C in a water bath until the TBA was completely dissolved, then transferred to a 100 mL flask, and the volume was filled up with distilled water.

❖ **Procedure**

Pipette into the glass vise test tubes 200 µl of sample and 800 µl of TBA reagent and close tightly. Heat the mixture in a water bath at 100 °C for 15 minutes. Then cool in a coldwater bath for 30 minutes, leaving the tubes open to allow the gases formed during the reaction to be removed. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer.

❖ **Expression of results**

The concentration of TBARS was determined using the molecular extinction coefficient of MDA (å = 1.53 105 M-1 cm-1). The results were expressed in µmol/1.

MDA (µmol/mg of t) = (OD sample /1.53 x 10^5) / mg of Tissue

2.3.3.3. Determination of reduced glutathione (GSH) level

❖ **Principal**

The level of reduced glutathione is determined according to Weckbecker & Cory, (1988). By measuring the optical density results from the formation of 2-nitro-5- mercocapturic acid (TNB) from the reduction of dithio-bis-2-nitrobenzoic acid (DTNB), which is called Ellman reagent with SH groups exist in GSH briefly.

❖ **Procedure**

800 µl of homogenate samples are added to 200 µl of salicylic acid (0.25%). Then centrifuged at 1000 rpm for 5 min.

Take 500 ml of supernatant and mix it with 1000 µl of tris buffer solution (tris 0.4 mol, NaCl 0.02 mol; ph = 8.9) and 25 µl of DTNB (0.01 mol/L).

Read the absorbance at λ = 412 nm after 5 minutes of incubation.

❖ **Expression of results**

$$GSH\ (nM/mg\ \ of\ protein) = \frac{(OD \times 1 \times 1.525)}{13133 \times 0.8 \times 0.5 \times mg\ \ of\ protein} \times d \qquad \text{mg of Tissue}$$

GSH concentration is expressed according to the following formula:

- **OD**: Optical Density.
- **1.525:** total volume of blend an ml.
- **13133**: Absorption constant of SH groups at 412 nm.
- **0.5:** volume of solution float an ml.
- **1:** volume of protein mixture.
- **0.8:** volume of homogeneous solution without protein exists in 1 ml.
- **GSH:** concentration of glutathione.
- **d:** dilution factor.

2.3.3.4. Determination of Super Oxide Dismutase (SOD) activity

❖ **Principle**

This test is based on the inhibition of NBT reduction by SOD. NBT is reduced by the superoxide anion O2-, and it is known that SOD neutralizes O2-, which inhibits the reduction of NBT (Beauchamp & Fridovich, 1971).

❖ **Procedure**

Collect in tubes	Blank	Sample
EDTA-Met (0.1mM, 13mM)	1000µL	1000µL
Phosphate buffer (50Mm)	892,2µL	892,2µL
Sample	-	50
Phosphate buffer (50Mm)	1000µL	950µl
NBT (75µM)	85,2µL	85,2µL
Riboflavin (2µM)	22,6µL	22,6µL

❖ **Expression of results**

Inhibition percentage of NBT reduction by SOD as follows:

IP (%) = (OD blank – OD sample) / (OD blank) ×100

2.3.4. Histological study

Histological sections were done following the method of Ishfaq et *al*., (2019), with a slight modification. 10% buffered formalin was used for fixing spleen, liver, heart, kindey samples, followed by dehydration with graded ethanol. The samples were processed for paraffin wax and cut into sections (5 µm thickness). After that, the sections were mounted on slides, stained with hematoxylin and eosin dye, and observed under a light microscope (10X and 40X magnifications).

2.3.5. Statistical analysis

The results obtained are expressed as the mean ± standard error of the mean (Mean ± SEM). The analysis of the data was carried out by application of the Student's T test. All data in this study were examined by Minitab 13.0 software. $p < 0.05$ indicates statistically significant difference.

Chapter II

Results

I. Results

1. *In-vitro* assays of copper oxide nanoparticles (CuONPs)

1.1. Characterization of CuNPs

1.1.1. UV–Vis spectroscopy

Notably, the change in color serves as the initial indicator of nanoparticle formation. Figure 01 displays the UV-Vis spectrum, showing a peak at 330nm, this peak is a characteristic feature of nanoparticle structures and further confirms the successful synthesis of copper oxide nanoparticles in the study.

Figure 11: UV-Vis spectra of copper oxide nanoparticles.

1.1.2. FT-IR analysis

The FTIR a CuO nanoparticle spectrum are Shown in figure04 broad representing the Association of different efficient groups in the Metal oxide NPs creation

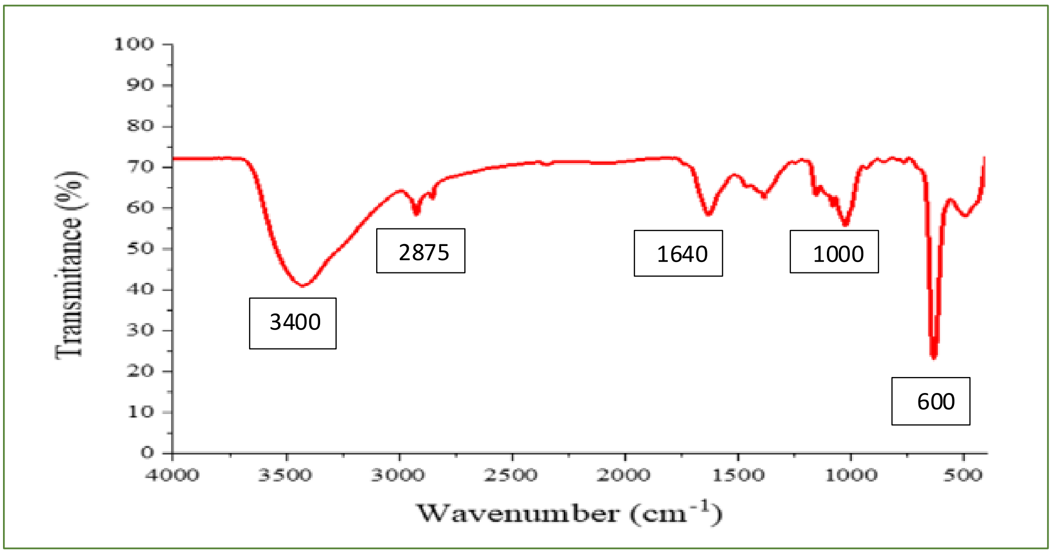


Figure 12 : Fourier infrared spectrum of sample

1.1.3. SEM and EDX studies

In Figure 3A, the scanning electron microscopy (SEM) image provides insight into the surface morphology of the nanoparticles, The surface is characterized by a coarse, porous, and irregular texture, consisting of non-uniform clusters of disparate size and morphology. This even distribution is a critical characteristic of nanoparticles. Figure 3 (B, C, D), on the other hand, displays the Energy Dispersive X-ray Spectroscopy (EDX) data, which indicates the elemental composition of the sample. It clearly confirms the presence of Copper and Oxygen, consistent with copper oxide nanoparticles. EDX results show the presence of the elements Zr, carbon, sulfur which indicates the presence of components oof the proteins bound to the elements of copper (Table 1)

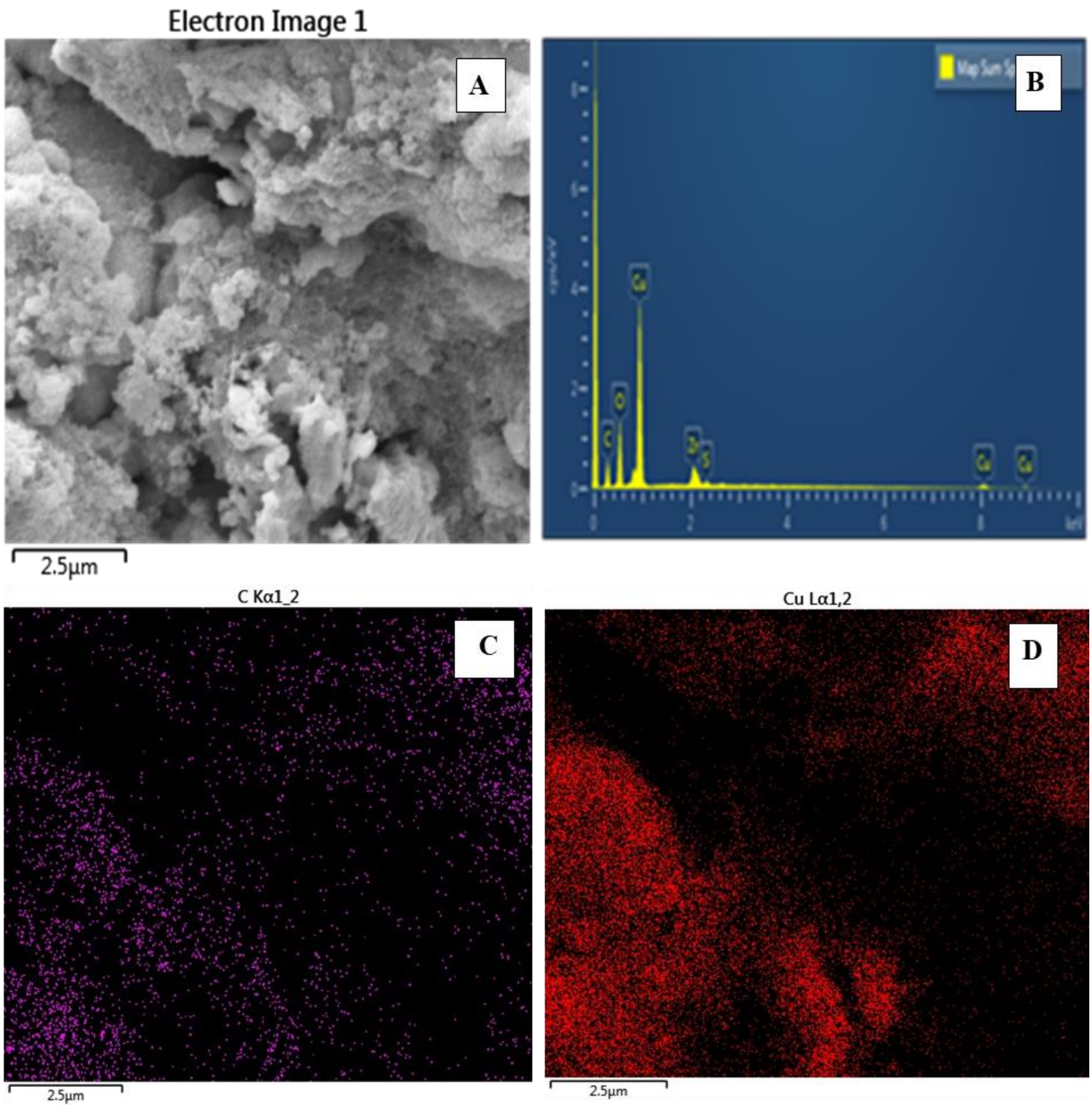


Figure 13: Scanning electron microscopy image and EDX of SV-CuNPs

Table 1: The elemental composition analyses of the CuNPs from the EDX polt of the SEM images SV-CuNP

Element	Line type	APParent Concentratic	K Ratio	Wt	Wt Sigma	Standard Label	Factory Standard	Standard Calibration Date
C	K serie S	0.01	0.00012	20.35	0.64	C Vit	Yes	
O	K Serie S	0.06	0.00022	15.82	0.33	SiO2	Yes	
S	K Serie S	0.00	0.00002	0.90	0.13	FeS2	Yes	
Cu	L	0.11	0.0011	58.76	0.81	Cu	Yes	
	Serie S		5					
Zr	Serie S	0.01	0.00010	4.17	1.02	Zr	Yes	
Total				100.00				

1.1.4. XRD studies

According to the XRD data that matched the JCPDS card Nº 00-074-1230 database, the chemical formula of the copper oxide nanoparticles was Cu_2O, and their crystallite structure was cubic. The lattice parameters were a = b = c = 4.25 Å, with a crystallite size (D) of 25.3 nm. The diffraction angles corresponded to the (110), (111), (200), (220) and (311) h, k, and l crystallite planes, respectively, with peaks at 29.67°, 36.56°, 42.52°, 61.59° and 73.81° (Figure 14).

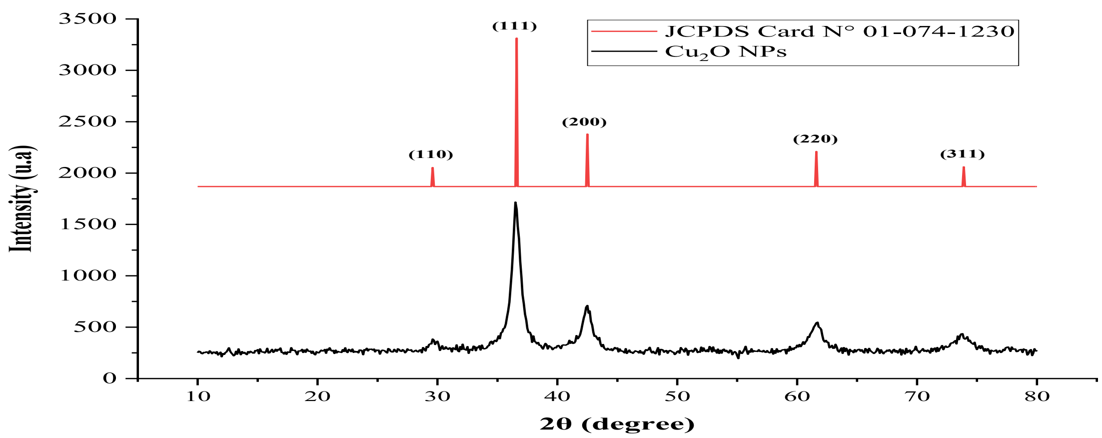


Figure 13: XRD patterns of CuO NPs.

1.1.5. TEM image

Transmission Electron Microscopy (TEM) is employed to depict the size and shape of the green-synthesized copper oxide nanoparticles bio-Synthesized copper nanoparticles created using Scorpion venom displayed size 100 nm. It appears that the particles are irregularly shaped, with some particles appearing spherical or subspherical and others being aggregated into larger clusters. as shown in Figure05(A). The size of the synthesized copper nanoparticle created using Scorpion venom was in the range between 3 and 10nm, as shown in Figure 05(B), with an average size 5.78±1.39nm. -Precise size measurement is vital for understanding the features of nanoparticles and their appropriateness for certain applications. It establishes their size distribution and consistency

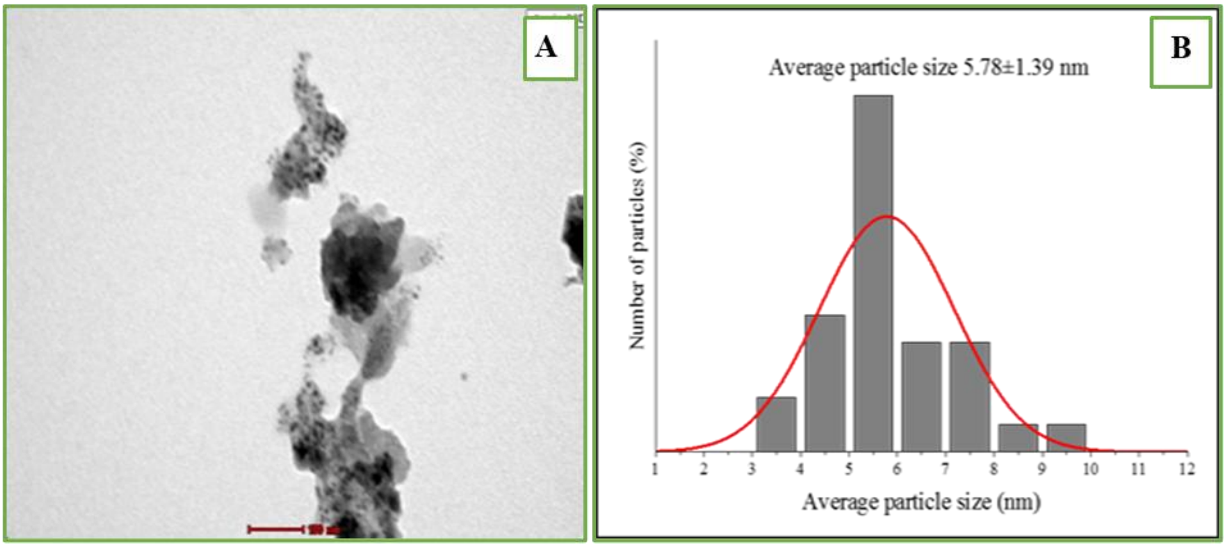


Figure 14: XRD patterns of CuO NPs.: TEM (A) and particle size analysis using histogram (B) of green synthesized copper oxide nanoparticles

1.1.6. Zeta potential

The zeta potential measurement for CuONPs synthesized using Scorpion venom yielded a value of approximately -32.59 mV, which indicates the electrical charge on the surface of the produced nanoparticles (Figure 16).

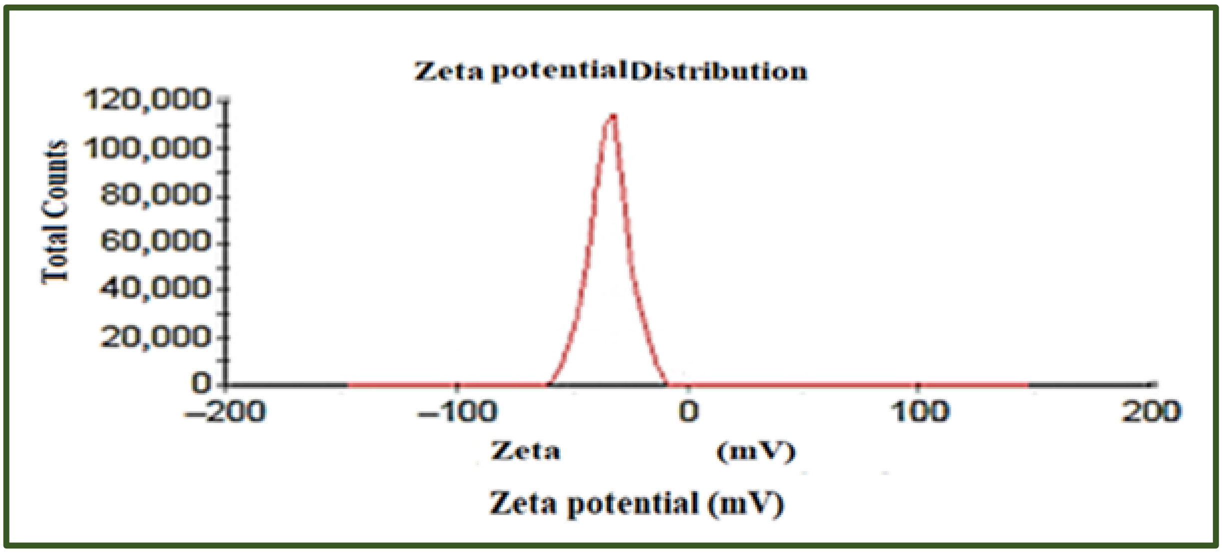


Figure 15: Zeta potential analysis of CuO NPs

1.1.7. XPS analysis

X-ray Photoelectron Spectroscopy XPS) is analysis on the Sv-CuNPs surface was conducted to understand the chemical state and composition, such as the oxidation state and chemical environment around Cu atoms.

Overall XPS spectrum of the Sv-CuNPs is shown in Fig. 1a. The individual element of core

level Cu 3p at BE value of 77 eV, Cu 3s at 122 eV, Cu 2p at 932.5 eV and 952.5 eV, Cu LMM at 570.5 eV, O 1s at 531.5 eV, and C 1s at 285 eV are observed.

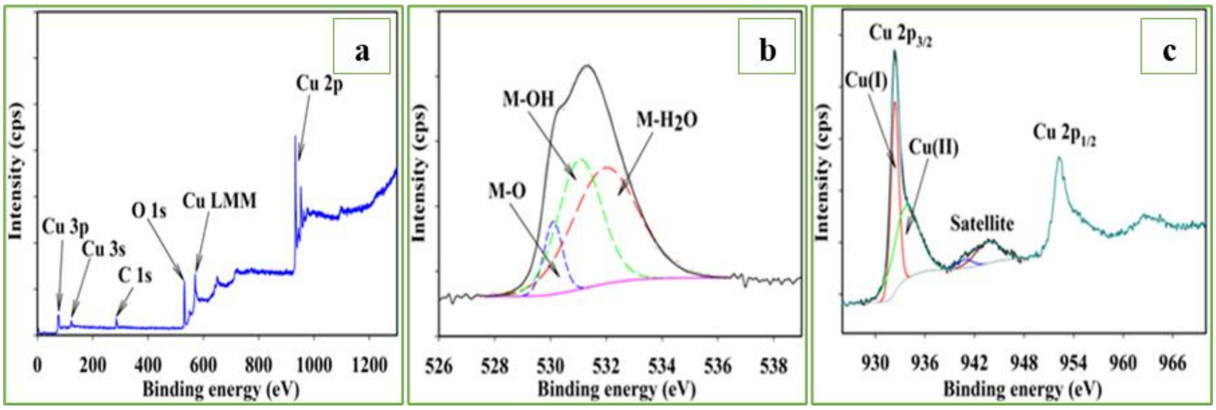


Figure 16: XPS analysis of SV-CuNPs surface (a) wide range scan (b) core level O 1s peak (c) core level Cu 2p peak

1.1.8. Disk Diffusion Assay

Assessment of Antimicrobial Activity: The antimicrobial effect of SV-CuNPs was evaluated Table 1 and figure 2 show the results of the inhibition zones for the strains: *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. We observed that the inhibition zone size for *Escherichia coli* was 23.5mm, while the inhibition zone size for *Staphylococcus aureus* was 6.3mm and for *Klebsiella pneumoniae* was 11mm. Regarding antibiotics, there was a difference between the strains *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. For the *Escherichia coli* strain, there was no effect of the antibiotic Ampicillin, while Gentamicin had a significant effect of 18mm. For the *Staphylococcus aureus* strain, Ampicillin was more effective, with an inhibition zone size of 39 mm, compared to Gentamicin, which had an inhibition zone size of 19mm. For the *Klebsiella pneumoniae* strain, Gentamicin had a significant effect, with an inhibition zone size of 19mm, compared to Ampicillin, which had an inhibition zone size of 7mm.These findings provide valuable insights into the efficacy of copper oxide nanoparticles in inhibiting microbial growth, and may inform the development of novel antimicrobial application

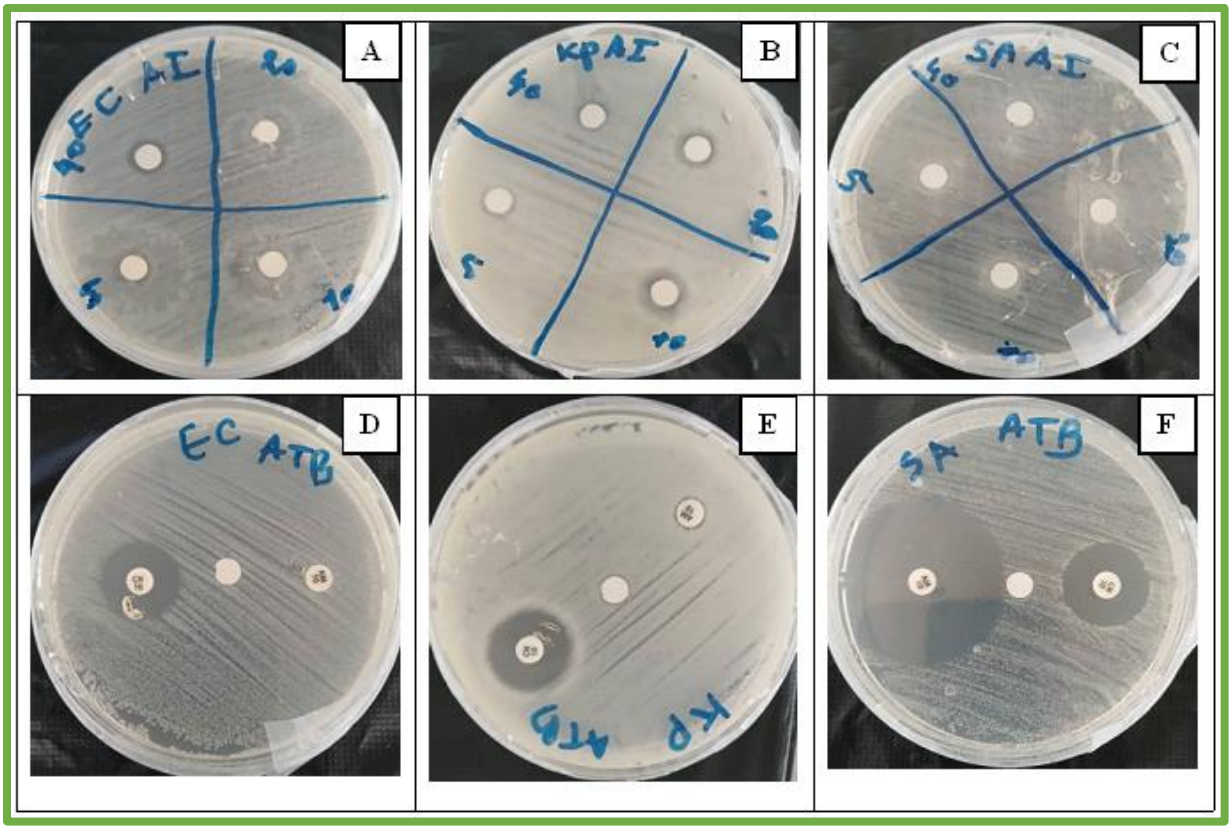


Figure 17: Zone of Inhibitions produced by SV-CuNPs (A-C), and Antibiotic (D-F) against different bacterial strains tested

1.2. Protein determination

The protein immobilization efficiency on the nanoparticles was found to be 30.92%, suggesting a relatively effective binding capacity and favorable interaction between the protein molecules and the nanoparticle surface.

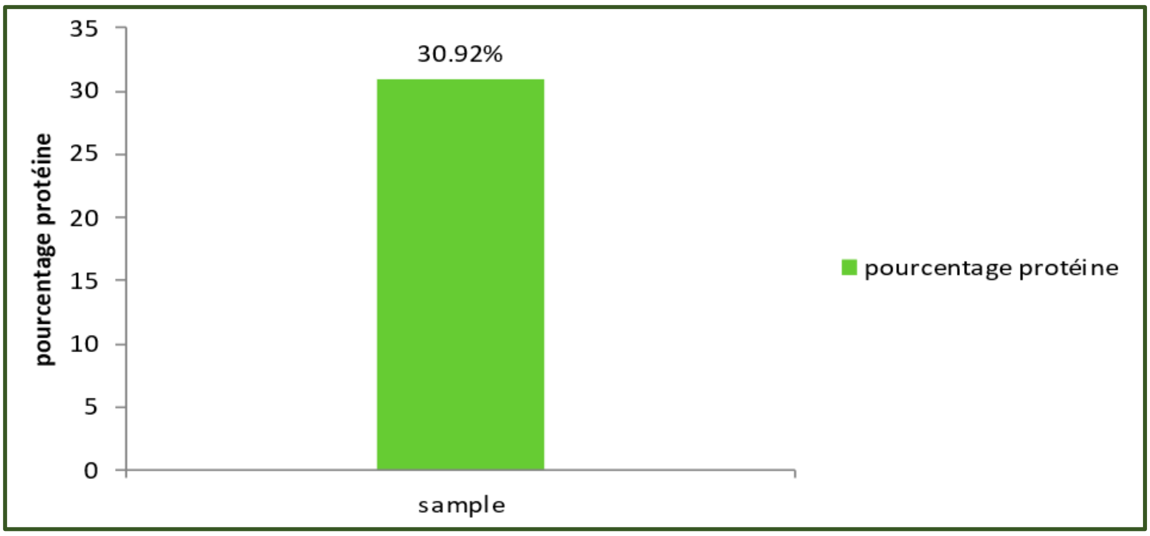


Figure 19: Protein rate in nanocomposites

II.2 In-Vivo study:

II.2.1. *In vivo* Acute Toxicity Evaluation of CuNPs

No mortality was noted on the acute toxicity test at any CuNPs dose tested. No symptoms of toxicity effects on behavioral and physiological parameters, including skin, eyes, diarrhea, and sleep were recorded during this period. All animals administered with any dose tested showed no signs of toxicity, their behavioral and physiological parameters were normal, and no mortality was recorded during the 14 days of the experimental period (Table).

Table 2: Mortality, physiological parameters and behaviour observations after acute toxicity using copper oxide nanoparticles

Parameter	Dose									
`Mortality	Control	/	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
	25 mg/ kg	/	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
Skin	Control	/	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)
	25 mg/ kg	/	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)
Eyes	Control	/	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)
	25 mg/ kg	/	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)
Diarrhea	Control	/	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
	25 mg/ kg	/	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
Sleep	Control	/	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
	25 mg/ kg	/	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %

(n): normal / 0 %: none

II.2.2. Growth parameters

Relative organ weights is summarized in table 3 of the three groups (SV-CuNPs+ SV,SV, and control), the results showed no change in the weights of the kidneys, heart, and spleen in the PSV While we noticed a significant increase in the liver group compared to the control group , while a significant decrease was observed in the weights of these organs, specifically the kidneys ($p<0.01$), spleen ($p<0.01$) and liver ($p<0.001$) in the SV-CuNPs+ SV group compared to the SV group, with a slight decrease in the heart ($p<0.05$).

Table 3: Growth parameters for control and experimental groups

Organ	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
Relative Kidneys weight (%)	0,6198±0,0264	0,6040± 0,0151 ^{Ns}	0,5377± 0,0328$^{**\ b}$
Relative liver weight (%)	1,729± 0,500	2,022± 0,496 ***	1,815±0,453^{***c}
Relative Heart weight (%)	0,3268± 0,0125	0,3440±0,0176 ^{Ns}	0,3145±0,0177 $^{Ns\ a}$
Relative Spleen weight (%)	0,2773±0,0208	0,2930±0,0110 ^{Ns}	0,2540±0,0144 $^{Ns\ b}$

Values are mean ± SEM. p<0.05*, p<0.01**, p<0.001***: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from infection **group (SV).** NS: No significance

II.2.3. Hematological parameters

The results of the hematological analysis of the SV groups showed a slight increase in WBC and Lymph ($p<0.05$), while GRAN no significant difference compared with the control group. In contrast, compared to SV-CuNPs + SV, we observed a slight increase in both WBC and Lymph ($p<0.05$), while showed that was no significant difference in GRAN (table 4).

Table 04: Leukocyte line in blood of control and experimental groups

Parameters	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
WBC (10^3/µl)	7,825±0,403	10,50± 1,00*	7,73± 1,18 $^{NS\ a}$
LYMPH (10^3/µl)	5,840± 0,175	8,10±1,00*	5,967±0,882 $^{Ns\ a}$
GRAN (10^3/µl)	1,800±0,235	1,600±0,227^{NS}	1,300±0,306 $^{*\ NS}$

Values are mean ± SEM. p<0.05*, p<0.01**, p<0.001***: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from infection **group (SV).** NS: No significance

Table 5 illustrates that, compared to the control, there was a significant ($p<0.05$) to a very high significant decrease ($p<0.001$) of red blood cells (RBC), hemoglobin (HBG), and hematocrit (HCT), with a slight increase in PLT ($P<0.05$), MCV($P<0.05$), MCH ($P<0.01$), and MCHC($P<0.01$) in the SV group. In the SV-CuNPs + SV group, the RBC, HBG, and HCT increased significantly high (p<0.01, p<0.01, p<0.001), while a very high decrease was observed in both MCV (p<0.001), MCH(p<0.001), and PLT(p<0.01), but no significant MCHC.

Table 5: Erythrocyte and Platelet line in blood of control and experimental groups

Parameters	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
RBC (10^6/µl)	7,2033±0,0726	6,3000±0,0723***	6,995±0,175 NS b
HGB (g/dl)	15,180± 0,092	14,400± 0,400*	15,750± 0,150** b
HCT (%)	37,600± 0,321	34,000±0,500**	37,750±0,250 NS c
MCV (fl)	52,133±0,088	56,25± 1,35*	53,200± 0,200**c
MCH (pg)	21,425±0,411	23,400± 0,436**	22,800±0,551*c
MCHC (g/dl)	41,74±1,16	42,150±0,222**	43,18±1,50 NS NS
PLC (10^3/µl)	888,00± 6,00	971,3±24,8*	817± 128** b

Values are mean ± SEM. p<0.05*, p<0.01**, p<0.001***: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from infection group (SV). NS: No significance

II.2.4. Immunoglobluin determination

The SV group showed a notable increase in IgA levels compared to the control group. This increase indicates a natural immune response resulting from exposure to the pathogen without vaccination intervention. It shows that the "SV-CuNPs+SV" group has higher levels of IGA compared to the SV group. This means that SV-CuNPs significantly enhances IGA levels, indicating a strong immune response.

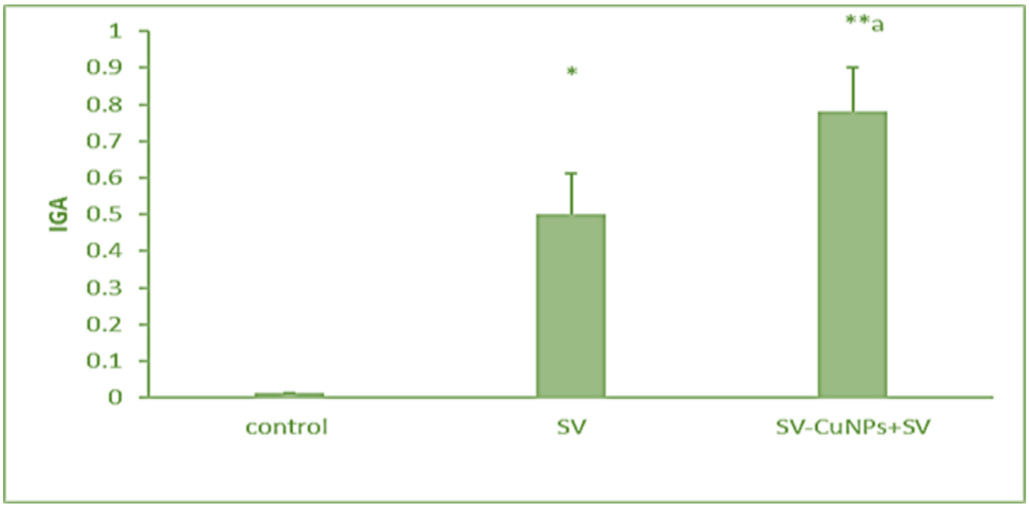


Figure 18: Immunoglobulin determination

II.2.5. Biochemical parameters

II.2.5.1. Blood sugar and lipid profile levels Cholesteroland, Triglycerid

The table 6, we observed that the levels of blood sugar tests showed incresed significantly high ($p<0.01$) for SV compared with the control group. In contrast, the group of SV-CuNPs + SV showed a slight increase ($p<0,05$) compared to SV group as for the results of the SV group of TC. They significantly increased ($p<0.01$) compared with the control group. While we observed a very highly significant increase ($p<0.001$) in the of SV-CuNPs + SV group compared to the SV group. For triglycerides results, we observed no change in the 2 groups, SV and SV-CuNPs + SV

Table 6: Blood sugar and lipid profil of control and experimental groups

Parameters	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
Gly (g/l)	59,75±5,65	78,50± 5,48**	68,00± 5,86 NS a
TC (g/l)	0.7767± 0.0555	0.9540±0.0581**	0.7100±0.0200**c
TG (g/l)	0.6725±0.0656	0.6520±0.0275 NS	0.6675±0.0229NSNS

Values are mean ± SEM. p<0.05*, p<0.01**, p<0.001***: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from infection group (SV). NS: No significance

II.2.5. 2. Urea, creatine and Uric acid

Table (7) represents the levels of creatine, urea, and uric acid for the experimental groups as shown a significant a very high increase ($p<0.001$) in the levels of creatine for the group SV compared with the control group. while we observed a very high decrease($p<0.001$) for the SV-CuNPs + SV group compared with non-vaccinated group. Urea levels showed a slight increase($p<0.05$) for group SV compared to control group, while the SV-NPs+ SV showed a slight decrease ($p<0.05$) compared to the SV group. Uric acid levels showed a slight increase ($p<0.05$) for the SV group compared to control group. while we observed a slight decrease ($p<0.05$) for the SV-CuNPs+ SV group compared to SV group.

Table 7: Urea levels and creatine activity in control and experimental groups

Parameters	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
Crea (mg/l)	6.175 ±0.419	7.625 ± 0.287***	7.0667±0.0882***c
Urea (g/l)	0.6633± 0.0260	0.7567±0.0706NS	0.6725±0.0592NS a
Uric Acid (mg/dl)	26.482± 0.364	30.10± 1.03**	27.410± 0.542*a

Values are mean ± SEM. $p<0.05$*, $p<0.01$**, $p<0.001$***: significantly different from control group. a $p<0.05$, b $p<0.01$, c $p<0.001$: significantly different from infection **group (SV).** NS: No significance

II.2.6. Enzymatic parameters

The following table (8) represent the results of ASAT and ALAT analysis, witch showed in the levels of both analyzes a significant increase ($p<0.001$) for group SV-CuNPs +SV vaccinated compared to SV group in contrast we observed non substantial difference ($p<0.05$) for the group non vaccinated compared to control group.

Table 8: ASAT and ALAT activities in control and experimental groups

Parameters	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
ASAT (UI/l)	71.0± 10.4	119.7± 23.8*	57.50± 5.36**c
ALAT (UI/l)	58.20±4.68	124.7± 24.8**	49.25 ±5.28*c

Values are mean ± SEM. $p<0.05$*, $p<0.01$**, $p<0.001$***: significantly different from control group. a $p<0.05$, b $p<0.01$, c $p<0.001$: significantly different from infection group (SV). NS: No significance

II.2.6. Oxidative stress parameters

II.2.6.1. Malondialdehyde (MDA) marker

In the analyzed tissues (table 9), MDA levels exhibited a significant increase in all organs for SV group compared to the control group: liver ($p<0.05$), heart($p<0.001$), kidney($p<0.01$), spleen($p<0.001$), The SV-CuONP+ SV group showed a significant decrease in MDA levels in the spleen and kidney ($p<0.001$, $p<0.05$), while we did not notice any change in the liver and heart compared to SV group

Table 9: Tissues MDA concentration in control and experimental groups

Organe	Control (n=5)	SV (n=5)	SV-CuNPs + SV (n=5)
Liver (nmol/g tissue)	6,127± 0,217	11,20±2,56*	7,94±2,19 NS NS
Heart (nmol/g tissue)	49,83± 1,22	55,684 ± 0,949***	48,529 ±0,098***NS
Kindey (nmol/g tissue)	45,67± 2,03	51,67± 2,33**	42,67±2,85 NS C
Spleen (nmol/g tissue)	39,8 ±11,8	60,30±1,13***	49,15±3,46* a

Values are mean ± SEM. $p<0.05$*, $p<0.01$**, $p<0.001$***: significantly different from control group. a $p<0.05$, b $p<0.01$, c $p<0.001$: significantly different from infection **group (SV).** NS: No significance

II.2.6.2. Reduced glutathione (GSH) activity

The results of GSH levels in the different tissues represented in the table 10 show that, as compared to the control group. There was a highly significant decrease in GSH levels in all tissus livers($p<0.01$); heart, ($p<0.05$); kidney ($p<0.001$) of the SV group but not in the spleen. Furthermore, the GSH increased significantly in the different tissus liver ($p<0.001$); heart ($p<0.01$); kidney ($p<0.001$) of the SV-CuNPs + SV group but not in the spleen compared with the SV group

Table 10: Tissues GSH concentration in control and experimental groups

Parameters	Control (n=5)	SV (n=5)	SV-CuNPs (n=5)
Liver (ηmol/g tissue)	0,1820±0,0273	0,1467± 0,0227*	0,1550±0,0110* **NS
Heart (ηmol/g tissue)	0,07533±0,00684	0,061±0,00186***	0,06800±0,00902 NS NS
Kindey (ηmol/g tissue)	0,147± 0,00837	0,141± 0,00250**	0,13250±0,00050***C
Spleen (ηmol/g tissue)	0,12175±0,00929	0,0977± 0,0118***	0,1597± 0,0203***b

Values are mean ± SEM. p<0.05*, p<0.01**, p<0.001***: significantly different from control group. p<0.05, b p<0.01, c p<0.001: significantly different from infection **group (SV).** NS: No significance

II.2.6.3. Superoxide dismutase (SOD) marker

Compared to the control group, spleen and heart SOD activities showed a very high significant decrease (p<0.001) in SV group, while a significant diminution in liver (p<0.05) and kidney (p<0.01) SOD activity. Furthermore, a significant decrease in the SV-CuNPs+ SVgroup decreased very high significant (p<0.001) in the kidney and a significant increase in the spleen (p<0.01); in contrast, no change any liver and heart compared to SV group table 11.

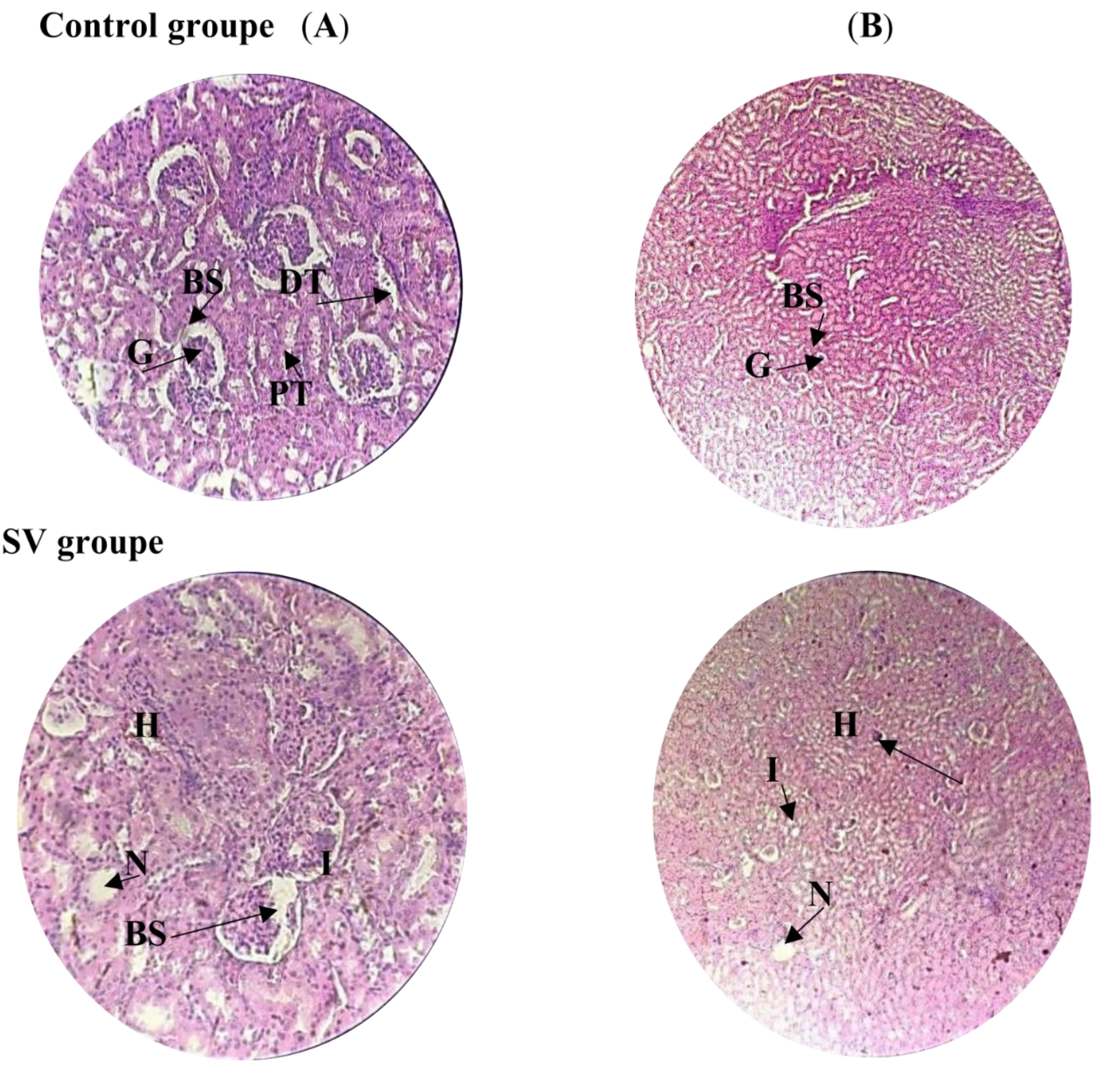
Table 11: Tissues SOD activity in control and experimental groups

Organe	Control (n=5)	SV (n=5)	SV-CuNPs+ SV (n=5)
Liver (U/g tissue)	0,8530± 0,0625	0,5830±0,079**	1,0360±0,0030*** c
Heart (U/g tissue)	0,5300± 0,0640	0,3837±0,0673*	0,5377±0,0592 NS b
Kindey (U/g tissue)	0,849±0,154	0,639± 0,191***	0,7900± 0,0516***c
Spleen (U/g tissue)	0,6517±0,0503	0,7390±0,0619 NS	0,7600±0,0586* NS

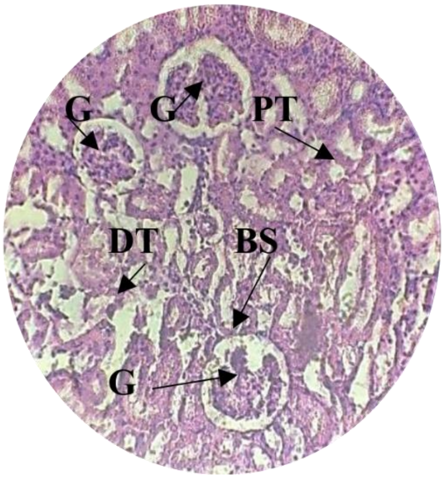
Values are mean ± SEM. p<0.05*, p<0.01**, p<0.001***: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from infection **group (SV).** NS: No significance

II.2.7. Histopathological examination results

The histological examination results in the two microscopic images show kidneys taken from mice representing three different groups. Microscopic examination of the kidneys in the control group reveals a normal renal cortex containing narrow glomeruli with limited Bowman's space, as well as regular proximal and distal convoluted tubules with central nuclei, without signs of inflammation or tissue damage. As a result of scorpion venom injection, the kidneys display marked histological changes, including dilated Bowman's space, capillary congestion, and infiltration of multinucleated inflammatory cells around the glomeruli and tubules, as well as tubular cell necrosis and structural distortion. In the group of mice treated with the SV-CuNPs+ SV, microscopic sections show a marked improvement in kidney structure, with more regular glomeruli and a near-normal Bowman's space. Renal tubules also feature a clear lumen and interstitial tissue devoid of dense inflammatory infiltrate, suggesting the vaccine's role in mitigating toxic effects and restoring structural balance to the kidney



SV-CuNPs+SV groupe



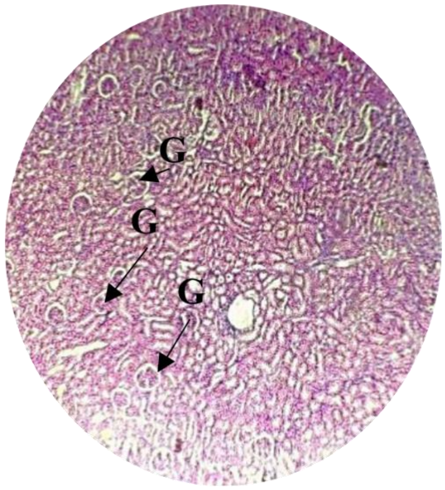
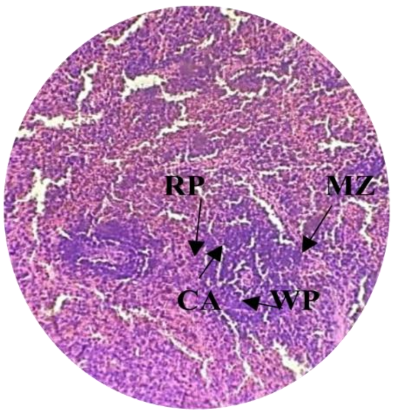


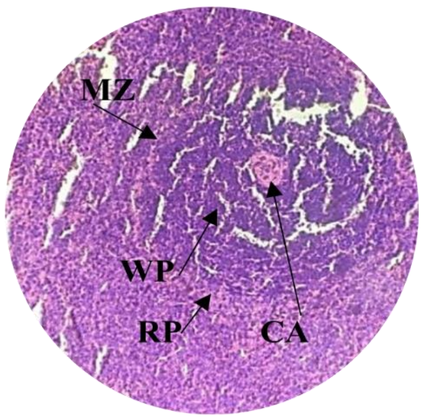
Figure 20 : Photomicrographs of kidney section of all experimental groups stained with hematoxylin and eosin.

(A): x10 and **(B)**: x40 **G**: Glomerulus, **BS**: Bawman's space, **PT**: Proximal tubules, **DT**: Distal tubules **I**: Inflammation, **H**: Hemorrhage, **N**: Necrosis

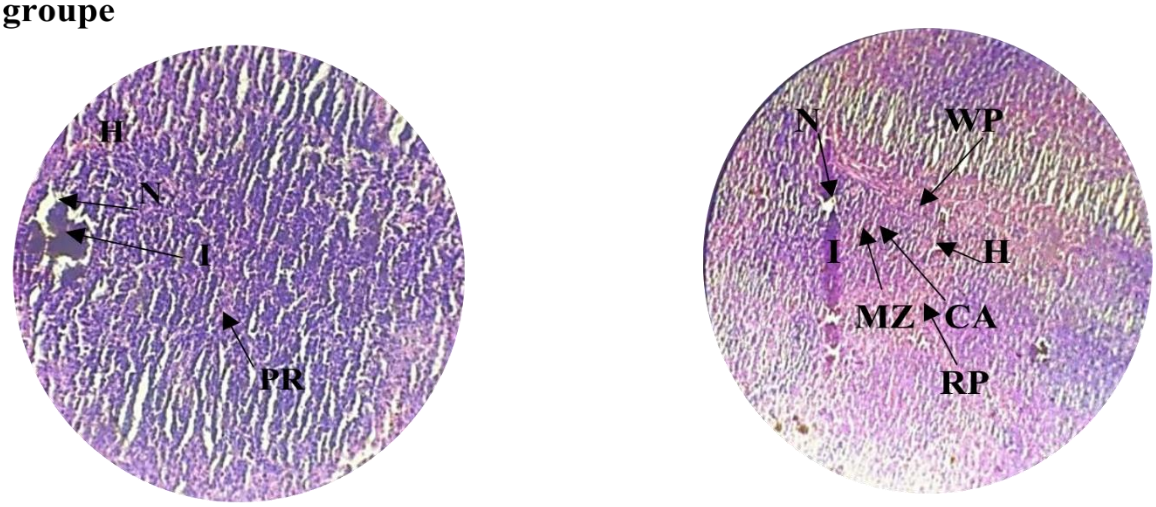
Histological analyses of the spleen in the control group showed a completely normal structure free from various deformities, consisting of two pulps, white pulp and red pulp, intermingled naturally. In contrast, spleen sections in the group affected by scorpion venom exhibited clear deformities in both pulps, with the white pulp appearing disorganized and losing the natural arrangement of lymphatic sheaths, and areas of edema and cell-free zones. Meanwhile, the red pulp was characterized by noticeable congestion and expansion of blood sinuses. These changes completely disappeared in the vaccinated group SV-CuNPs+ SV), where the spleen regained its natural structure with the presence of active lymphoid follicles in the white pulp and the regular arrangement of capillaries in the red pulp, indicating an effective immune response against the venom (Figure 20).

Control groupe (A) **(B)**





SV groupe



SV-CuNPs+SV groupe

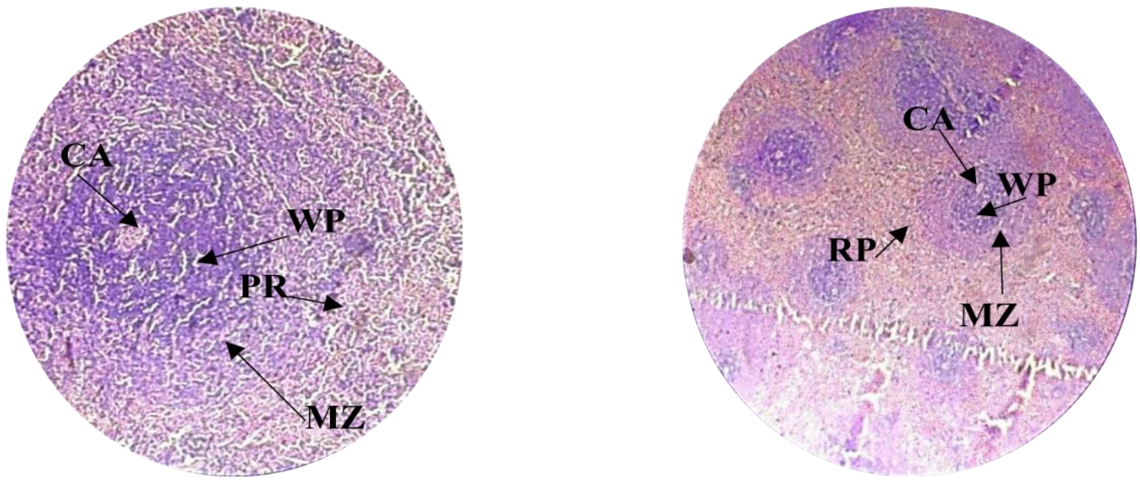


Figure 21: Photomicrographs of spleen section of all experimental groups stained with hematoxylin and eosin**.**

(A): x10 and **(B):** x40.**RP:** Red pulp, **WP:** White pulp, **CA:** central arteriole, **MZ:** Marginal zone **I:** Inflammation, **H:** Hemorrhage, **N:** Necrosis

Histological analyses of the liver in the control group showed a normal tissue structure, with hepatocytes arranged radially around the central veins, with clear round nuclei and group, where we observed a relatively healthy liver structure, with only mild immune open blood sinuses, without signs of degeneration or cellular infiltration. However, liver sections in the scorpion venom-treated group showed signs of tissue damage, including immune cell infiltration and partial disorganization of hepatocytes, with signs of cellular degeneration in some areas, compared to liver sections in the control group, which did not show any of these changes. These changes completely disappeared in the SV-CuNPs+ SV infiltration around the vessels, demonstrating the vaccine's effectiveness in protecting against the toxic effects of scorpion venom without causing obvious tissue damage.

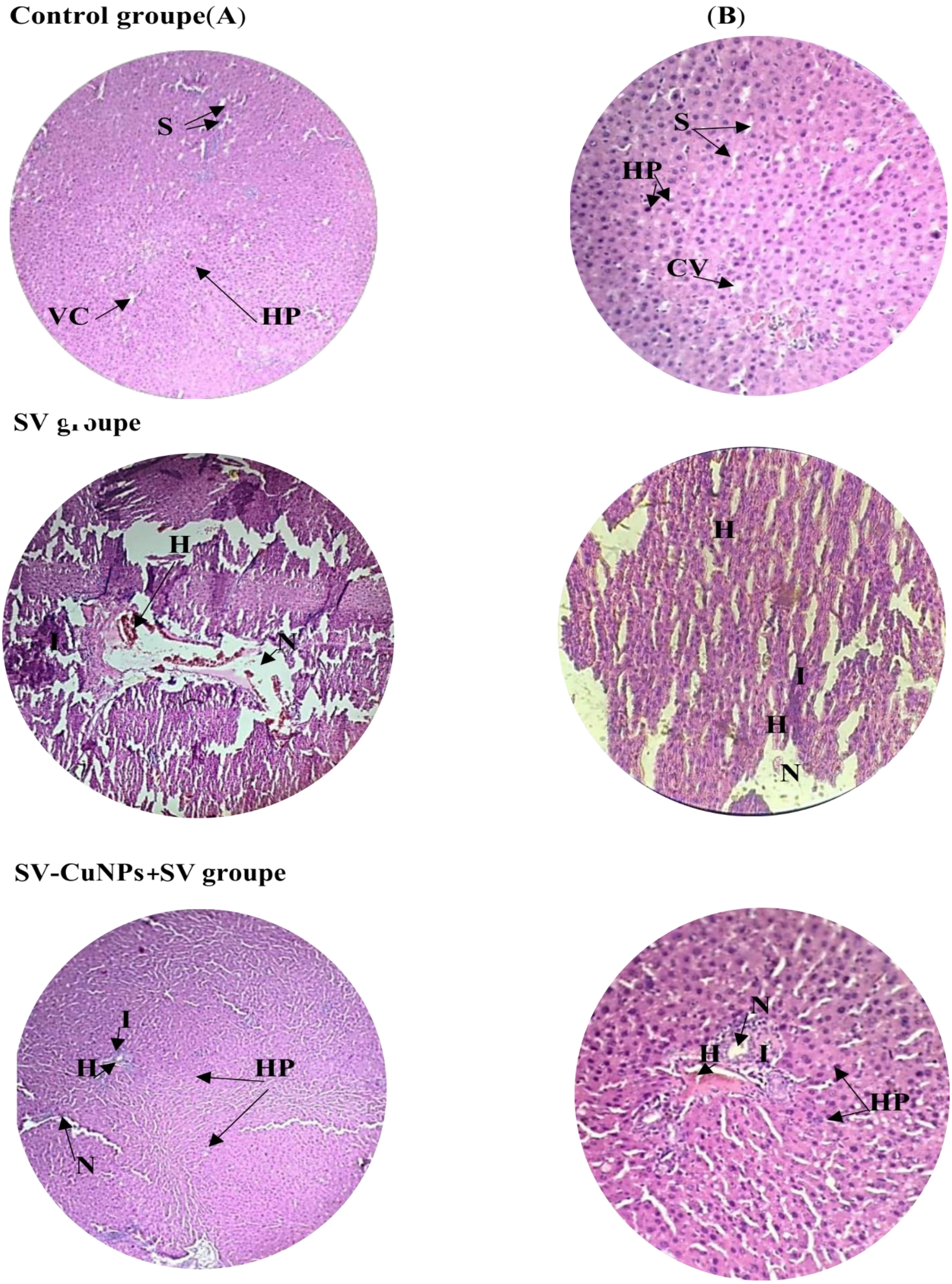


Figure 22: Photomicrographs of liver section of all experimental groups stained with hematoxylin and eosin.

(A): x10 and **(B):** x40 Hp: hepatocytes **S:** sinusoids **CV:** central vein **H:** hemmorage **I:** inflammatory **N:** necrosis

Chapter III

Discussion

III. Discussion

Venomous creatures, such as scorpions, provide potential for innovative medication treatments with minimal side effects. Scorpion venoms have been shown to effectively treat life-threatening human parasites such as Plasmodium, Leishmania, and Trypanosoma (Jafari et *al*., 2019). Scorpion venoms include several pharmacological peptides, which have piqued scientists' interest in medication development. (Perumal et *al*., 2017). Envenomation (SE) is a public health issue in poor areas. In Algeria, the population at risk of SE was expected to be 86.45% in 2019. Thus, developing a vaccine to protect exposed populations against scorpion poisons would be a significant step forward in the fight against this illness (Benazzouz et *al*., 2024).

1. Characterization of nanoparticles

Copper oxide nanoparticles was conducted using UV-Vis Spectrophotometry, the change in colour was the first indication of Formation of nanoparticles. The sharp peak at 330 nm during UV-Vism8k, Our UV-visible spectroscopy results are consistent with prior research by Punetha et *al*., 2022. who found that the ultraviolet-visible spectrum of copper oxide nanoparticles for increased bioactivity was 330 nm.

Fourier Transform Infrared spectroscopy, this shows how efficient groups play a part in making metal oxide nanoparticles. The C−O and OH-bunches on the CuO crystal nanostructure surface mostly absorb in the 2800-4000 wavenumber cm−1 range. The broad absorption peak at 3431 cm-1 is generated by adsorbed water molecules in nanocrystalline materials, which absorb moisture due to their high surface-to-volume ratio, as previously studied by Radhakrishnan et *al*., 2014. CuO nanostructures exhibited an infrared absorption peak between 400-600 cm^{-1} in their vibrational modes. The peak at 584 cm^{-1} represents the production of CuO nanostructures by the earlier studies of Vinod et *al*., 2013. The bands at 1645 cm^{-1} and the peak at 1629 cm^{-1} show the carbonyl C=O stretching bonds. Thus, the C-H stretching bonds are represented by the area 3300-2800 cm^{-1} in accordance with the previous studies of Xu et *al*., 2007. The exact process that causes CuO NPs to develop within cells by confronting starch with parallel metal ions remains unknown. Sugars found in starch may function as a capping and reducing agent in the production of metal oxide nanoparticles. Perhaps metal ions create metal nuclei, which then elevate and aggregate in the form of nanoparticles within the starch are similar to the previous studies of silva et *al*., 2003. The FTIR data can support these assumptions.

Scanning electron microscopy showed that nanoparticles created using Scorpion venom were aggregated and the presence of elemental copper was confirmed by EDX. SEM revealed nanoparticle diameter (Ray et *al*., 2015). In their study, Prabhu et al. discovered that even the tiniest copper nanoparticles were hazardous (Prabhu et *al*., 2010). The results show that starch was encapsulated on the particles. Copper nanoparticles collect and agglomerate when distributed in deionized water, as suggested by previous research (Prabhu et *al*., 2010; Song et al., 2014). The TEM analysis picture revealed relative spherical NPs that ranged in size from 3 to 10 nm. as previously studied by Mali et *al*., 2020.

The dimensions of CuNPs produced in this work suggest their potential for biotechnological uses at acceptable concentrations. X-Ray Diffraction pattern confirmed the successful synthesis of copper nanoparticles created using Scorpion venom from starch; wherein the main diffraction peaks characterize the elemental copper were detected at 20 = 29.67, 36.56, 42.52, 61.59, and 73.81, which correspond to the (110), (111), (200), (220), and (311) crystal faces of copper and compared with the standard powder diffraction card of JCPDS, cop-per file No. 00-074-1230 Berra et *al*., (2018).The cubic crystal structures of Cu_2O closely match the standard as JCPDS Card No.00-074-1230 Thakar et *al*., 2022).The measurement of zeta potential for CuO NPs created using Scorpion venom revealed a value of about -32.59 mV, indicating the electrical charge on the nanoparticles' surface. Van der Waal's forces of attraction cause nanoparticles to tend to aggregate when they are dispersed in water. Nevertheless, if the particles become charged during dispersion, aggregation might be avoided if the electrostatic repulsive forces outweigh the Van der Waal's attractive forces (Ponticorvo et *al*., 2022). Zeta potential measurements are used to evaluate the surface characteristics of nanoparticles by characterizing the charge distribution on their surface (Mansouri et *al*.,2018).The negative or positive charge on metal nanoparticles contributes to their long-term stability and prevents aggregation (Chetehouna et *al*., 2024). The development of hydroxyl groups on the particle surface during dispersion in water may be the cause of the negative zeta potential value. The adsorption of hydroxyl groups on the surface of particles is encouraged by raising the pH, which raises the zeta potential (Devadoss et *al*., 2023).

XPS analysis on the SV-CuNPs surface was conducted to understand the chemical state and composition, such as the oxidation state and chemical environment around Cu atoms.

Overall XPS spectrum of the SV-CuNPs is shown in Fig. 1a. The individual element of core level Cu 3p at BE value of 77 eV, Cu 3s at 122 eV, Cu 2p at 932.5 eV and 952.5 eV, Cu LMM at 570.5 eV, O 1s at 531.5 eV, and C 1s at 285 eV are observed. Cu 2p peak has two distinct peaks due to spin-orbit splitting (j-j coupling) nature where Cu $2p_{3/2}$ has higher intensity than Cu $2p_{1/2}$ (with the typical ratio of 2:1) but with position at lower binding energy. Shake-up satellite peaks are also detected due to multiple splitting, plasmon loss or shake-up processes(Conradie & Erasmus, 2022). No other elements are detected, affirming the absence of contamination on the CuNPs surface. Fig. 1b exhibits core level O 1s peak which comprises with three individual components. O 1s at 531.9 eV for M-H_2O (surface adsorbed water molecules), 531.0 eV for M-OH (hydroxyls) and 530.1 eV for M-O (lattice oxygen), respectively(Jiang et *al*., 2021). The % ratio of these oxygen components is found to be 9.2 % for M-O, 38.8 % for M-OH and M-H_2O for 52%. Fig. 1c displays the core level Cu 2p peak. It comprises two main peaks with binding energy centered at 932.3 eV for Cu $2p_{3/2}$ and 952.2 eV for Cu $2p_{1/2}$. Two shake-up peaks are also detected at binging energies of 943.7 eV and 962.6 eV, respectively. From the deconvoluted result, mixed valent Cu species are observed in the core level Cu 2p peak. Two components for Cu $2p_{3/2}$ binding energy centered at 933.7 eV and 932.4 eV, which can be suggested as Cu (II) and Cu (I) states and Cu $2p_{1/2}$ at 943.7 eV for Cu (I) and 940.8 eV for Cu (II)(Khoo et *al*., 2020; Zhen et al., 2019). Furthermore, the presence of shake-up satellite peaks confirms the Cu^{2+} nature (Khalakhan et *al*., 2021).

The antibacterial activity was performed using the disk diffusion method (MWITARI et *al*., 2013). It was revealed that the effect of copper oxide nanoparticles depends on the particle size, the structure of the bacterial cell wall, and the degree of bacterial cell compatibility to determine the bactericidal effect of different immobilization processes for attaching microorganisms to the nanoparticles (Liang et *al*., 2012) The greater sensitivity of both bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, to copper oxide nanoparticles is attributed to the template of copper oxide nanoparticles, as well as the higher abundance of carboxyl and amine groups on the bacterial cell surface, which increases the attraction of copper ions to these groups according to the study of le cref et *al*., 1990. Different methods have been proposed to translate the antibacterial effects of metal oxides. Pearlstein et *al*., 2009 reported that in the case of copper oxide coated fabric, an antibacterial effect was found due to reactive oxygen species produced by copper oxide nanoparticles, which are responsible for the destruction of bacterial cells (Azam et *al*., 2012).

2. Hematological parameters

Our results demonstrated a significant increase in the leukocyte line in SV group compared with the control. The majority of immunological cells are thought to be leukocytes. These cells provide complete monitoring to many organs and tissues via the normal circulation of blood to lymph, from the inside of the arteries to the outside, and from the inside of the tissues to the blood, resulting in the body's defense against pathological elements. In general, leukocytes are divided into three types of cells: lymphocytes, monocytes, and granulocytes (Vodjgani, 2012). Scorpion venom activates the immune system, causing enhanced oxidative and inflammatory stress responses. (Tobar et *al*., 2024) found that venom greatly increased the inflammatory response in plasma samples. Adjuvants are co-immunized with vaccine antigens, which aids in the induction of a strong antigen-specific immune response by increasing antigenic exposure time at the injection site or activating antigen-presenting cells via pathogen recognition receptors (PRRs), resulting in improved antigen exposure to T lymphocytes (immune potentiators). Although adjuvants have been known for about 90 years, only a handful are successfully employed, with alum being the most prevalent (Nanishi et *al*., 2020). Venom-associated molecular patterns bind to TLR-2 and TLR-4 on both blood leukocytes and endothelial cells, inducing the creation and release of pro-inflammatory and anti-inflammatory cytokines. An effective adjuvant would generate the necessary signals for efficient T cell activation and immune activator production, such as cytokine secretion, reactive oxygen or nitrogen intermediates, PRR stimulation, and upregulation of costimulatory molecules in antigen-presenting cells. Due to various characteristics, scorpion venom can be a powerful immunological stimulant. The venom is mostly constituted of neurotoxins, which can stimulate the creation of nerve growth factor. Immune cells such as macrophages, B cells, and T cells are known to possess NGF receptors via which they are controlled (Skaper, 2017). Such cross-talk between the neurological and immune systems has an impact on the survival and multiplication of B and T cells (Torcia et *al*., 1996). Furthermore, clinical trials are being conducted to investigate the immunomodulatory impact of NGF in adjuvant formulations. Second, scorpion venom induces acute stress, a type of danger signal that activates the hypothalamus-pituitary-adrenal (HPA) axis, potentially eliciting an antigen-specific immune response when administered alongside the target antigen (Santhosh et *al*., 2016). Scorpion venom proteins are known to interact with innate and adaptive immune cells via their ion channels (Ramirez-Bello et *al.,* 2014; Feske et *al*., 2015). For example, the venom of Androctonus australis hector has been demonstrated to target K+ channels in macrophages, which are critical in the activation and proliferation of immune cells leading to cytokine production (Vicente et *al*., 2003). Furthermore, scorpion venom is known to have immunomodulatory effects. The venom stimulates macrophages, releasing TNF-α and increasing lymphocyte migration to the peritoneal cavity (Petricevich and Lebrun, 2005; Maciel, 2014). Previous research has demonstrated that scorpion venom peptides are identified by PRRs (TLR2, TLR4, CD14) and cause the production of signaling molecules such as cytokines, reactive oxygen, and nitrogen species (Zoccal et *al*., 2014; Khemili et *al*., 2020). Previous research indicates the venom triggers the release of neuroendocrine factors (acetylcholine and corticosterone), activating immune cells and releasing cytokines (IL-1β, IL-6, IL-10, and TNF-α) into the bloodstream (Santhosh et *al*., 2016). In vitro studies have also demonstrated that scorpion venom activates T lymphocytes (Casella-Martins et *al*., 2015).

The use of toxins as an adjuvant or carrier protein is not novel. A well-defined innate immune response can determine the scope and size of the adaptive immunological response. Certain innate immune cells, including antigen-presenting cells, help activate antigen-specific immune cells in the spleen, lymph nodes, and peritoneum (Chaplin, 2010). The spleen and peritoneal cavity are made up of many immune cells such as macrophages, neutrophils, dendritic cells, and B and T cells, the composition of which changes throughout illness (Cassado et *al*., 2015; Lewis et *al*., 2019).

In contrast, the group immunized by SV-CuONPs+ SV showed a significant decline in the leukocyte line parameters group compared with the infection group. Vaccines are likely to mimic illnesses, inducing a primary immune response that sets the framework for a secondary immune response when exposed to the actual pathogens. Giesker & Hensel (2014) argue that the secondary immune response is distinguished by its speed and potency, allowing for more efficient pathogen clearance than the initial response. Marshall et al. (2018) emphasize that immunologic memory is a critical component of adaptive immunity. This memory gives the host the ability to develop a quick and strong immune response upon re-exposure to the antigen, so strengthening the body's defense systems. Ademokun and Dunn Walters (2010) explain that subsequent exposures with the same antigen cause a secondary response in both B and T cells, activating pre-existing memory cells more quickly.

Nanovaccines, which contain NPs (as delivery/ material), have piqued the interest of scientists and the health sector since they have been shown to selectively stimulate humoral and/or cellular immune responses. Beyond the traditional antibody-inducing effects of vaccines, the involvement of the cellular immune response, as reflected by the activation of CD8+ T cells that directly destroy infected or aberrant cells, has lately been highlighted in vaccinations against viral infections and cancer. Furthermore, it has been widely recognized that several nanovaccines induce cellular immunity, particularly CD8+ T cell responses (Nakamura & Harashima, 2017). On the other hand, it is never too much to underline the necessity of understanding vaccination functioning principles in order to improve their safety and efficacy while also limiting their negative effects (Pulendran & Ahmed, 2011). DCs, one of the most effective professional antigen-processing cells (APCs), are necessary for bridging innate and adaptive immunity via antigen absorption, as well as processing and displaying epitopes on naive T cells. To elicit adequate CD8+ T cell responses to an exogenous antigen, DCs transmit epitopes from the antigen to CD8+ T cells via a technique known as "cross-presentation" (Joffre et *al*., 2012; Gros, 2019). Because most vaccines used in the field are exogenous to the cell, DCs play an important role in vaccine-induced activation of cytotoxic T lymphocyte (CTL) responses against viral or neoplastic illnesses. As a result, many nanovaccine methods have been developed to target DCs (Roopngam, 2019; Mumper et *al*., 2003). Studies have been conducted to determine the method of action of nanovaccines by concentrating on intracellular components and their functions. Novel nanovaccines aimed to generate protective CTL responses have been proposed and developed as a result of accumulating proof-of-concept investigations on cross-presenting.

Our findings showed a substantial drop in red blood cells in the SV group compared to the control group. Consistent with our findings, this venom had a strong effect on red blood cell hemolysis in both vitro and in vivo (Mirakabbadi et *al*., 2007)

Protein poisons from animals, plants, and bacteria can cause hemolysis, especially in marine species (Parker & Feil, 2005). Several of these venoms damage biological membranes by causing the development of holes or channels in real and model bilayer lipids Membranes (García-Sáez et *al*., 2011; Savva et *al*., 2013). Thus, hemolytic activity is generated by protein toxins. has been employed as a sensitive toxicological tool. Investigate protein targeting and attachment cellular membranes (Sabirov et *al*., 1993). scorpion venom caused hydrophilic holes in the erythrocyte cell membrane. produced colloid osmotic burst, resulting in erythrocyte. lysis. In addition to pore-forming processes, the lipid peroxidation of erythrocyte membranes plays a significant part in the hemolysis caused by hemolytic protein. Toxins cause cell membrane dysfunction (Parker & Feil., 2005 ; García-Sáez et *al*., 2011)

Platelets, derived from bone marrow megakaryocyte cells, play a critical role in blood hemostasis and coagulation. Activated platelets also contribute to host defense mechanisms (Dwaya et al., 2022). With a size of 2–5 µm and no nucleus, platelets rely on cytoplasmic RNAs, ribosomes, and mitochondria to operate. They have two different kinds of granules: dense granules that enhance mood by containing calcium, serotonin, and nucleotides α-granules, which contain chemicals that promote hemostasis and platelet activation immunological processes such as CD40 ligand and von Willebrand factor (Scherlinger et *al*., 2023). We see an increase in platelet count after scorpion envenoming, which contradicts the previous study (Bechouni, n.d.), which indicated a fall in platelet counts. The platelet count drops over the course of 24 hours until it returns to normal. Our research indicates that scorpion envenomation has a clear hematological effect on hemostasis in the human bod.

In the context of our study, our results showed a decrease in HGB and HCT, whereas an increase in MCV, MCHC, and MCH was observed in the SV groupe compared to the control group. According to a study by (Emam et *al*., 2008), it was found that decrease in hemoglobin level among their patients. This was attributed to the action of scorpion venom on the red blood cell membrane causing hemolysis. While we observed a significant decrease in HCT and an increase in both MCH and MCHC, these results are consistent with the study of (Costal-Oliveira et *al*., 2015) except for MCV, which slight increased. This is the opposite of his study.

A study revealed that the SV-CuNPs combination significantly boosts Immunoglobulin A (IgA) levels, confirming its effectiveness in stimulating mucosal immunity. This finding is crucial as IgA plays a key role in protecting mucosal surfaces from infection. These results, although from an animal study, align with previous human studies. For instance, a study by Gorochov et al., (2024) involving 427 individuals who received mRNA vaccines (Moderna or Pfizer-BioNTech) found that vaccination increased salivary IgA levels, particularly in individuals not previously infected with SARS-CoV-2. Additionally, previously infected individuals showed a stronger and more sustained mucosal immune response. This consistency in results across different models (animal and human) and vaccine technologies (nanoparticles and mRNA) suggests that an IgA-mediated mucosal immune response is a reliable indicator of vaccine efficacy, regardless of the platform used. This supports the development of future vaccines aimed at enhancing mucosal IgA, especially against viruses that enter the body via mucous membranes like SARS-CoV-2.

3. Biochemical and enzymatic parameters

We observe in our study hyperglycemia upon scorpion envenoming, and this is consistent with the study that states. Hyperglycemia is frequently seen in severely scorpion-envenomed individuals. It is caused by a strong autonomic storm, which includes a huge release of catecholamines, elevated glucagon and cortisol levels, and either low insulin levels or insulin resistance. Hyperglycemia indicates the severity of this disorder. Hyperglycemia has been linked to the severity of clinical symptoms of severe scorpion envenomation (Bahloul et *al*., 2018)

Scorpion envenoming leads to increased cholesterol levels after 60 minutes (Mirakabbadi et *al*., 2007), consistent with our findings of elevated cholesterol in the SV group versus controls. Notably, The SV-CuNPs+SV group showed a significant decrease in cholesterol levels compared to the SV group.

The frequency of scorpion envenomation complaints grows year after year. The uncontrolled spread of dangerous scorpion species, as well as the growth of metropolitan areas, contributes to an increase in scorpion encounters (Ahmadi *et al.,* 2020; Ward *et al.,* 2018). Every year, around 1 million scorpion envenomations occur, resulting in nearly 3250 fatalities (Cid-Uribe *et al.,* 2020). It was recently revealed that scorpion stings cause a range of symptoms, the most serious of which are cardio-respiratory dysfunctions (Sifi *et al.,* 2017), bleeding (Shah *et al.,* 2018*)*, and even local tissue necrosis (Jenkins *et al.,* 2021*)*. It is believed that the intensity and systematicity of scorpion envenomation are directly connected to venom neurotoxicity effects, namely neuronal stimulation and catecholamine release (Al-Asmari *et al.,* 2016; Isbister& Bawaskar, 2014; Ortiz *et al.,* 2015*)*. However, the development of severe systemic symptoms may be linked to increased enzymatic activity inside the tissues, which also promotes inflammation. Which also activates inflammation response, (Minutti-Zanella *et al.,* 2021; Petricevich, 2010)

In this study, we documented levels of biochemical and enzyme parameters (urea, creatinine, ASAT, ALAT, and uric acid). We observed a significant increase in levels of urea, creatinine, cholesterol, and uric acid. This is similar to this study (Salman & Hammad, 2017) which found that... (Blood and liver tissue samples were harvested 1, 2, and 4 hours after injection. Levels of cholesterol, creatinine, urea, and uric acid were significantly elevated in poisoned animals within 1, 2, and 4 hours after injection.) As well as increased levels of AST and ALT, this is similar to a study (Pipelzadeh et *al*., 2006), which found that after scorpion venom exposure, levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ALT lead to an increase in liver function.

According to our study, we observe an increase in these parameters when comparing the SV group to the control group.

4. Oxidative stress parameters

In this study, we observed a decrease in malondialdehyde (MDA) levels in all organs of rats injected with scorpion venom, which is in contrast to the previous study. They found that venom administration inhibited the increase in serum malondialdehyde (MDA) levels (Khemili et *al*., 2020). MDA is a biomarker that indicates antioxidant status, lipid peroxidation, and oxidative stress (Wang et *al*., 2021; Marino et *al*., 2023). It also raises pro-inflammatory cytokines (Mueangson et *al*., 2023). we also examined the SV-CuNPs+SV group and found a decrease in MDA levels in the spleen and kidneys, with no notable changes in the liver and heart compared to the control group. Macrophages have a role in both homeostatic and pathogenic processes, as well as complicated interactions with other cells (Stein & Gordon, 1991; Mosser, 2003). When exposed to venom, macrophages produce mediators such as nitric oxide (NO) and cytokines.

During phagocytosis, neutrophils engulf pathogens within phagosomes and initiate a respiratory burst by activating both phagocytic membrane NOX complex and NADPH oxidase. This activation leads to the generation of superoxide anions (O2−), which are subsequently converted into hydroxyl radicals (OH·), singlet oxygen (1O2), and hydrogen peroxide (H2O2) by superoxide dismutase (SOD). H2O2 serves as a substrate for myeloperoxidase (MPO), resulting in the production of hypochlorous acid (HOCl), a potent antimicrobial agent (Rajashekaraiah et *al*., 2021; Dagah et *al*., 2024).

The results of GSH levels in various tissues indicate that, compared to the control group, there was a highly significant decrease in GSH levels in all tissues: liver, heart, and kidneys, in SV-CuNPs+SV group. This finding is similar to a previous study that found venom administration led to a decrease in glutathione (GSH) levels in tissue homogenates. (Khemili et *al*., 2020)

There is very little known regarding the role of circulating glutathione levels in scorpion envenoming. Glutathione is a key reducing agent in tissues. Oxidized glutathione (GSSG) is damaging to tissues, particularly RBC. Glutathione (GSSG) is transformed into glutathione (GSH), which is necessary for the integrity of the RBC membrane. Glutathione, as a coenzyme with glutathione-insulin-transhydrogenase in the liver, aids in insulin catabolism and breakdown. Glutathione protects several SH group-containing enzymes against oxidation of the SH groups. Glutathione functions as a "co-enzyme" for the enzyme glyoxylase, which .transforms methylglyoxal to lactic acid (Radha Krishna Murthy et *al*., 2003).

Superoxide dismutase (SOD) protects against oxidative stress by removing potentially hazardous free radicals (Abdulgafor et *al*., 2018; According to Fodouop et *al*., 2015). We observed a decrease in SOD activity in all organs in the SV group (scorpion venom group), which is consistent with a previous study suggesting. Superoxide dismutase levels in poisoned rat were considerably lower than those in the control group at 1, 2, and 4 hours after injection. We may infer that crude venom causes oxidative stress in poisoned mice by inhibiting antioxidant mechanisms and affecting several metabolic markers (Salman et *al*., 2017).

5. Histological analyzes

A histological examination of the liver, spleen, and kidney organs was performed after rats were poisoned with a sublethal dosage of scorpion venom. Injecting rats with a sublethal dosage of scorpion venom resulted in morphological alterations and structural abnormalities in all investigated organs. The study found that spleen sections from the SV group had medullary anomalies intermingled with an increase in white medulla volume, which caused constriction and shrinking of the red medulla. This indicates that B and T lymphocytes have different distributions and proportions in the medulla (Rosche et *al*., 2015). Our findings showed liver tissue injury and cytoplasmic membrane loss. This investigation verifies the observation of necrosis and bleeding (Adaika et *al*., 2021). Scorpion venom increased vascular permeability in the liver (Lamraoui et *al*., 2015). Our findings revealed significant histological alterations in the kidneys, as well as degenerative changes in the tubular epithelium of the renal cortex. The proximal and distal tubules were enlarged and confluent. The lumen of the tubules was no longer distinguishable. The glomeruli seemed totally disordered. This is consistent with the findings of (Adaika et *al*., 2021) These pathological alterations were distinguished by hemorrhagic edema, necrosis, and the presence of inflammatory cells (Lamraoui et *al*., 2015).

This was also demonstrated in a study by Guo et *al*., (2024), who found that spleen sections from the SV-CuNPs+SV group had an intact structure, implying that lymph nodes (LNs) are good targets for nanovaccines to stimulate immune cells, particularly B and T cells capable of eliminating pathogens in the body. A nanovaccine's capacity to induce helper T cells 2 to create cytokines and plasma B cells to make antibodies against the vaccine determines the generation of particular antibodies against it. At this point, the nanoparticles and recombinant antigens generate the antibodies generated by the nanovaccine. Nanovaccines activate B cells in plasma cells to create specific antibodies against antigens attached to the nanoparticles (Bagheri et *al*., 2022).

Conclusion

❖ Conclusion

- ✓ The preparation of copper nanoparticles using starch represents one of the modern and innovative methods in developing nanomaterials. The development of copper nanoparticles using scorpion venom is a notable scientific achievement.
- ✓ The results showed that these nanoparticles possess a precise nanoscale size, which enhances their effectiveness and potential applications in medical and industrial fields.
- ✓ The body's immune system can activate a positive immune response towards the nanobio-vaccine composed of scorpion venom and copper, providing effective protection against harmful effects and supporting the body's immune defenses.
- ✓ Studies have shown that the impact of copper nano-vaccine and scorpion venom on hematological and biochemical parameters leads to notable improvements in overall health, enhancing the effectiveness of potential medical treatments.
- ✓ Copper nanoparticles integrated with scorpion venom exhibit strong protective effects against oxidative stress in the body, contributing to enhancing overall health and protecting against cellular damage.
- ✓ The scorpion venom vaccine shows strong protective effects against severe histological changes caused by the venom, contributing to protecting tissues from damage and promoting healing and recovery.

❖ Perspectives

- ✓ Exploring the role of SV-CuNPs as adjuvants to stimulate a stronger and more effective immune response against scorpion venoms.
- ✓ Using CuNPs as smart carriers for antigens extracted from scorpion venom to ensure their effective delivery to immune cells.
- ✓ Designing the vaccine based on the most common and dangerous scorpion venoms in the target region.
- ✓ Using advanced nano-synthetic techniques: integrating CuNPs with polymers or biological materials to form a smart vaccine that can be released on demand.
- ✓ Examining the stability and efficacy of treatments under different temperatures and humidity conditions, particularly in desert areas where scorpions are prevalent

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Annexes

Figure 01: Ultrasonic bath

Figure 02: Centerfuge

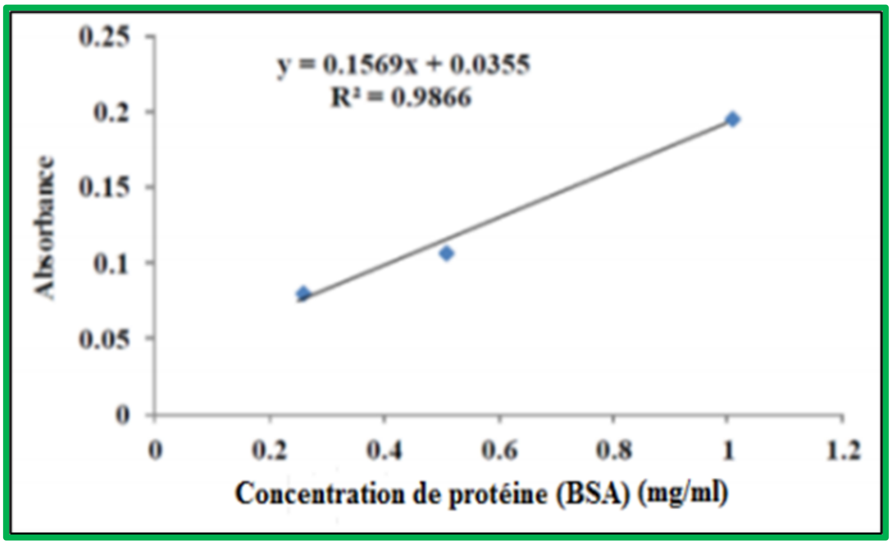

Figure 03: Protein calibration curve